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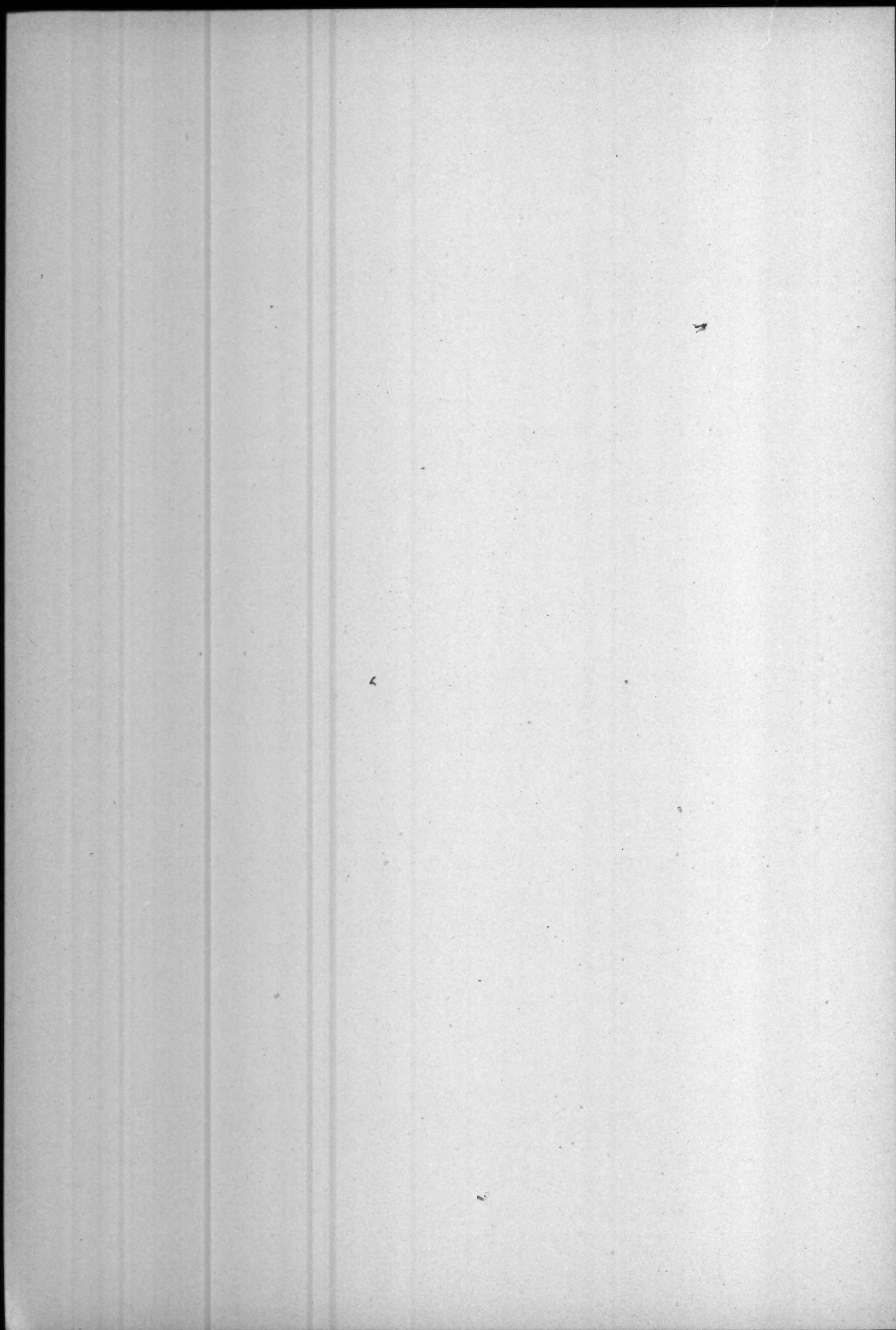
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BLURRING OF RETINAL IMAGE AND FOVEAL DIFFERENTIAL LIGHT THRESHOLDS*†

KENNETH N. OGLE, PH.D.
Rochester, Minnesota

The literature affords a number of papers dealing with the problem of the change in differential light threshold of the eye which occurs with a change in the size of the test details. But of these papers almost none deal also with the effect of blurring of the retinal image (by being made out of focus) upon those thresholds. Ronchi¹ did publish some data on the effect of blur with plus lenses; but because of the particular experimental arrangement used, his data are difficult to interpret.

A previous report² has described the increase in differential light threshold for foveal vision of a nearly point light source (angular diameter of 0.6 minute of arc) that results from progressively greater blurring of the retinal image effected by throwing it out of focus. As an extension of that study, data will be reported here that show the influence of blur on the foveal perception of circular test light disks of angular diameters from 0.6 to 20 minutes of arc, seen upon a white mat background whose luminance is about 12 millilamberts.

These data on detectability of the test disks were obtained under carefully controlled experimental conditions that excluded all variables except the degree of out-of-focus blurring of their images. Binocular vision was maintained for its stabilizing influence on accommodation by a background of wall charts, though the differential threshold of the disk was measured by the right eye only.

INSTRUMENTATION AND PROCEDURE

A haploscope was adapted for the study (see previous paper² for details). Figure 1

* Read at the meeting of the association for Research in Ophthalmology, New Orleans, Louisiana, December 6, 1960.

† This investigation was supported in part by Research Grant B-1637 from the National Institutes of Health, Public Health Service.

illustrates the principal features of the instrument. The subject saw the stimulus image by reflection from a semi-reflecting mirror and through an achromatic field lens mounted on the arm of the haploscope. The principal focal point of this lens coincided with the center of the entrance pupil of the subject's right eye. This arrangement of field lens and test target was based upon that of the well-known optometer of Badal.^{3,4} When the test object coincided with the second focal point of the field lens, the image of the test object as seen by the subject was at an infinite distance. The dioptric distance, Q diopters, was zero. At some particular position of the test object, u_0 , the dioptric distance of the image was the same as that of the wall charts (0.25 diopter). For positions nearer or farther than this particular distance u_0 , the dioptric distance of the image was nearer or farther than the wall charts; and the retinal image then was blurred from being out of focus. The degree to which the image was out of focus, ΔQ diopters, was equal to $-F^2(\Delta u)$, where F is the power of the field lens (diopters) and (Δu) is the displacement of the target (in meters). Thus the degree of out-of-focus blurring was related linearly to the displacement of the test target. Displacements of the target toward the lens (proximal) produced out-of-focus images on the retina corresponding to those of uncorrected hyperopia, while those away from the lens (distal) produced out-of-focus images like those in uncorrected myopia.

More important than this linear relationship are the facts that the angular size of the perceived image was the same and the light flux entering the fixed pupil of the eye was constant, irrespective of the degree to which the image was out of focus.

The luminous energy passing through a

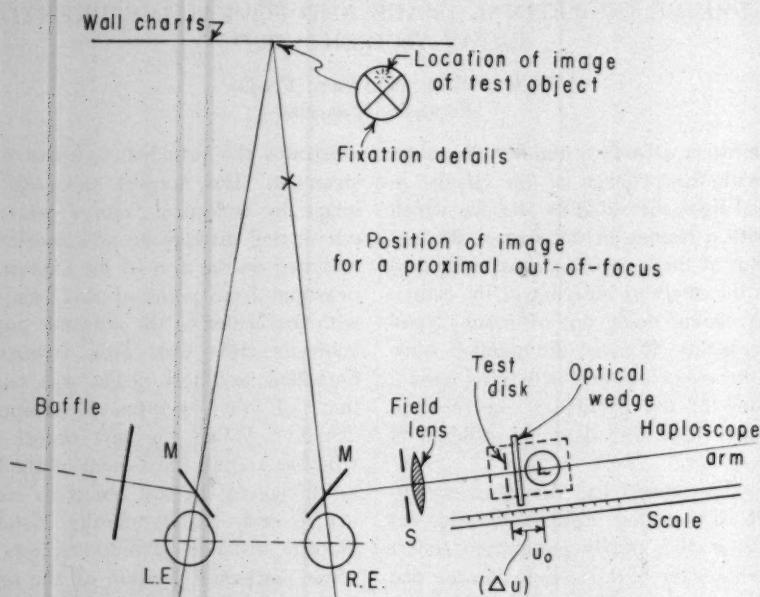


Fig. 1. (Ogle) Plan of experimental arrangement by which differential light-contrast thresholds were determined for test disks of different sizes and for out-of-focus retinal imagery.

given disk was provided by a ribbon filament lamp, and this could be controlled by a calibrated circular optical neutral-density wedge. The viewing distance was 4 meters. The length of the exposure of the test target visible to the subject was controlled by a photographic shutter, *S* (suitably monitored), in front of the field lens. For the data reported here the exposure of the test target was 0.50 second.

The instrument was so arranged that when the position of the head of the subject was adjusted to it and the shutter was opened, the subject saw the exposed image of the test disk slightly above the intersection of two diagonal crosslines on the central wall chart. This was the area fixated by the subject, and consequently foveal vision was used.

The procedure was to determine the luminous intensity of the test disk (white filament light) for which its image would just be visible above the luminance of the wall charts. This was determined with differ-

ent sizes of disks and with a series of different degrees of out-of-focus blurring of the images. Accordingly, the operator would set the position of the test target at one of a series of given values of (ΔQ) . When a given setting was made, the subject, on signal, fixated slightly above the intersecting lines on the wall chart and then exposed the light disk by operating the cable release to the shutter. He then answered "yes" or "no" as to whether he had seen the stimulus. The operator, altering the light flux passing through the disk by means of the circular density wedge, could determine by the method of limits the 50-50 threshold of visibility of the subject. The thresholds were determined alternately for increasingly proximal and distal out-of-focus imagery. Three subjects were used.

RESULTS

As found in the previous study,² the more the dioptric image on the retina of the test disk is thrown out of focus, the higher is

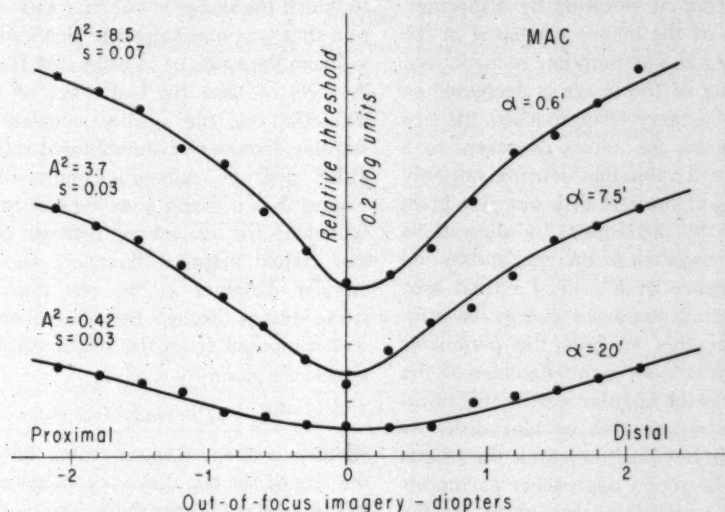


Fig. 2. (Ogle) Graphic illustration of increase in differential light threshold for disks of three angular sizes, as image on retina was made out of focus by fixed steps.

the differential threshold. This is to say, the more blurred the retinal image, the more intense must be the luminance of the test details to be detectable above the general luminance of the adapting field. With larger test objects, however, the required degree of increase in threshold luminance of the test object is less.

Typical graphs representing the results of one observer for three angular sizes of the test disk are shown in Figure 2. In this the abscissa corresponds to the dioptric value of the out-of-focus imagery—proximally to the left, distally to the right. The ordinates are the measured relative luminous intensities of the test disk for differential threshold, expressed in logarithmic units. It is evident that as the image on the retina is increasingly blurred, the differential threshold is increased; but the amount of this increase for a given degree of blurring is less as the size of the test disk is made larger. Thus, with blurring of the retinal image corresponding to one diopter, for a disk of angular size 0.6 minute of arc the differential threshold must be increased 0.75 log unit (5.6 times) above the threshold for sharpest

imagery; but for one 20 minutes of arc, the required increase is only 0.33 log unit (2.1 times). It will be noticed again that the threshold for proximal blurring is slightly lower than that for distal blurring.

The curves used to describe these data were calculated from a relationship essentially the same as that developed in the previous paper,² namely

$$\log B/B_0 = \log [1 + A^2(\Delta Q)^2 / (1 - s(\Delta Q))]. \quad (1)$$

In this, B is the luminous intensity required of the test disk to provide a differential threshold when the image is blurred to the extent of (ΔQ) diopters, and B_0 is the intensity for the lowest threshold when the imagery is sharpest;* A^2 is a constant dependent upon the angular size of the test disk and the pupil size, and s is a constant that describes the asymmetry between the thresholds for distal and proximal blurring of the retinal image. It is clear that the curves described by this relationship fit the data points satisfactorily.

The constants A and s were determined

* Actually B and B_0 would be proportional to the luminous energy entering the eye.

empirically from curve-fitting by inspection. On the basis of the theory presented in the previous paper the assumption is made that as the blurring of the image is decreased so that sharpest imagery is approached, the size of the image on the retina decreases to a minimal value, a value that depends not only upon the size of the test disk but also upon the limits set by diffraction, by aberrations of the dioptric system of the eye, and by the possible existence of a critical retinal area within which all luminous energy is summed. Under this analysis the parameter $A = 2r/\alpha_m$, where $2r$ is the diameter of the pupil and α_m is the angular size of this minimal effective retinal area or blur disk. As the size of the test disk increases, the stimulus area on the retina approaches asymptotically a line parallel to that given by the angular size of the test disk. This relationship of course depends upon the fact that the luminous flux entering the pupil of the eye is constant, irrespective of the degree

to which the image is out of focus—a condition that was maintained in this experiment.

From the value of A estimated for each of the sets of data for test disks of different diameter, one can readily calculate α_m , the angular diameter of the minimal retinal area (blur disk or summative unit, or both). Figure 3 is a graph showing the typical relationship for one subject between this minimal retinal angular diameter, α_m , and the angular diameter of the test disk, α . The curve drawn through these calculated points was computed from the relationship for the hyperbola given by

$$(\alpha_m - a)^2 - (m\alpha)^2 - c^2 = 0. \quad (2)$$

In this, a is the limiting value of α_m when the size of the test disk approaches zero; and c is a constant. From these results it appears that the minimal effective sizes of retinal area (the value of α_m when α becomes small, namely $\alpha_m|_0$) were about 6.1, 5.8, and 7.7 minutes of arc for the three subjects; and

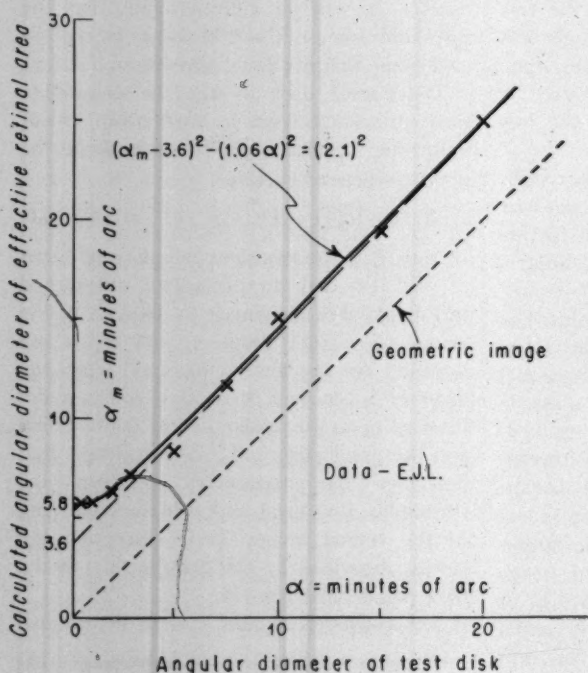


Fig. 3 (Ogle) Relationship between angular diameter of minimal effective retinal image and angular diameter of test disk, as determined by application of equation (1) of text.

TABLE 1
SUMMARY OF PARAMETERS ESTIMATED FROM CURVE-FITTING PROCEDURES

Subject (diam. pupil, mm.)	Equation (2)		Equation (3)							
			Imagery out of focus, diopters							
			0.00		0.60		1.20		1.80	
	$\alpha_{m 0}$	$\bar{\alpha}$	α_e	p^2	α_e	p^2	α_e	p^2	α_e	p^2
K.N.O. (4.8)	6.1	3.0	5.0	0.015	6.7	0.045	14.0	0.08	20.0	0.12
E.J.L. (5.3)	5.8	3.6	4.7	0.018	7.5	0.065	13.7	0.11	21.0	0.15
M.A.C. (6.0)	7.7	3.2	4.9	0.010	6.9	0.060	13.0	0.08	21.5	0.13
Average	6.5	3.3	4.9	0.014	7.0	0.057	13.6	0.09	20.8	0.133

$\alpha_{m|0}$ = limiting value of angular diameter of minimal effective retinal area, as size of test disk approaches zero.

$\bar{\alpha}$ = critical angle of test disk, minutes of arc.

α_e = critical visual angle of test disk, minutes of arc.

p^2 = constant, describing departure of data from asymptotes.

the critical angles α of the size of the test disk for the same were 3.0, 3.6, and 3.2 minutes of arc, respectively. These results could be taken to indicate that although the critical angles for the size of the test disk were about 3 minutes of arc, the actual minimal effective retinal area had angular diameters of about twice these sizes. The results of the curve-fitting in graphs such as Figure 3 and the subsequent calculation for the three subjects are shown in the table.

Of special interest are plots of the data to illustrate, for several degrees of blurring, the measured relationship between the differential light threshold and the angular size of the test disk. A typical example for one subject is Figure 4, the data being the mean of the proximal and distal thresholds for the same degree of out-of-focus blurring of the images. In this graph the angular diameters of the test disk are plotted (as is usual) along the abscissa on a logarithmic scale and the measured differential thresholds on the ordinate in logarithmic units.

There is a characteristic break in the course of the data for sharp imagery ($\Delta Q = 0$), as in those reported by Dreyer.⁵ This graph shows that, for test

disks below a critical size, the log differential light threshold increases linearly with a decrease in the log angular size. For test disks of increasing size above this critical angle the thresholds tend to a constant value. With sharp imagery, the critical angle for the subject whose data are illustrated was about 4.5 minutes of arc.

This result is consistent with the theory that for test disks smaller than a certain critical size, the size of the effective area of the image on the retina, whether due to optical or retinal factors or both, is constant. The luminous energy necessary to evoke a visual response in this area would be constant. But the luminous flux from the test disk would vary with its area. Thus the measured contrast threshold would have to increase inversely with the square of the diameter of the test disk. This, of course, is Ricco's law. On this log-log graph, lines drawn through the data points in the region of smaller test disk sizes have a slope of -2 , which is equivalent to a statement of Ricco's law.

As the size of the test disk becomes larger than the critical angle, the differential light threshold tends to depend only upon the

illuminance (areal density of luminous energy) on the retina, and therefore approaches a constant limiting value. This point of view is consistent with the experience that the brightnesses of different large areas having the same luminance tend to be the same.

Depending upon the experimental technique used, most sets of data reported (except that of Dreyer²⁰) do not show a really sharp break of the two branches of the data at the critical angle, but rather a more continuous curve. With the log-log method of plotting the data used here, a curve for a given set of data may be considered to be a portion of a hyperbola, the two branches approaching asymptotes. These asymptotes would be given by

$$\log \frac{B}{B_0} + 2 \log \frac{\alpha}{\alpha_c} = 0 \quad (\text{Ricco's law})$$

and

$$\log \frac{B}{B_0} = 0 \quad (\text{constant threshold})$$

in which, as before, B is the luminous flux from a test disk of angular diameter α necessary for the differential light threshold above the luminance of the background, B_0 is the same for the minimal differential threshold, and α_c is the critical angular diameter of the disk.

The hyperbola would then be given by

$$\left(\log \frac{B}{B_0} \right)^2 + 2 \left(\log \frac{B}{B_0} \right) \left(\log \frac{\alpha}{\alpha_c} \right) - p^2 = 0 \quad (3)$$

The smaller the constant p^2 , the more closely the curve hugs the two asymptotes, and hence the more sharp and apparent the break in the course of the data at the critical angle.

The curves drawn to represent the four sets of data in Figure 4 were computed from this equation, the values of B_0 , α_c , and p^2 being ascertained from curve-fitting by inspection.

On the basis of dioptrics and diffraction, one would expect that however small the test disk may be, the optical image on the retina is never smaller than a certain limiting size (for example, the diffraction disk).

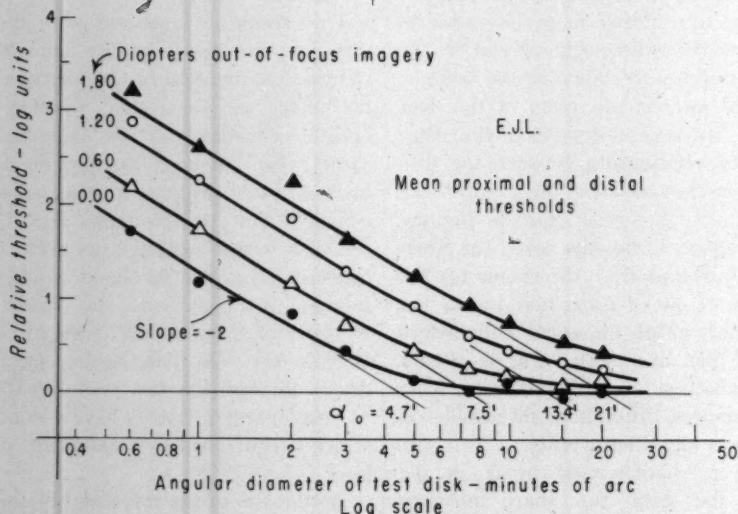


Fig. 4. (Ogle) Relationship between differential threshold and angular size of test disk, for imagery out of focus by different diopters.

On this basis alone the differential light thresholds for these smaller disks would be inversely proportional to the light flux from the disks, and therefore to their areas. This is the condition expressed by Ricco's law. However, this limit of retinal image size on the basis of optics and sharp imagery for a pupil of average diameter is estimated to be about 1.25 minutes of arc. This is only a small fraction of the angle suggested by the data given here.

Instead of considering the limiting area as being due to dioptric factors alone, we may also consider that there exist in the retina "quasi-independent" retinal areas (Baumgardt⁶) within which all incident luminous energy on the retina is totally summated to provide the visual response. In this situation also the asymptotic condition expressed by Ricco's law could be obtained, but with a larger retinal image.

The presently reported study was concerned primarily with the influence of blurring of the retinal image by throwing the dioptric image out of focus. An inspection of Figure 4, which presents data typical of all three subjects, shows that for a given degree of out-of-focus blurring the courses of the data follow the same pattern. For angles of less than a certain critical size the data obey Ricco's law. For larger sizes of the test disk the data approach the same horizontal asymptote as for sharp imagery. This means that for larger test disks the differential threshold is independent of the blurredness of the dioptric image on the retina. The critical angle, α_c , however, increases with the amount of blur, and the value of the constant p^2 increases slightly. The table summarizes also the values of α_c and p^2 computed for the three subjects from data plotted as illustrated in Figure 4.

The increase in the size of the critical angle with blurring of the retinal image suggests that under threshold conditions the

factor that sets the limiting size of the effective retinal area is primarily optical. But this implies that the blurred images on the retina are much larger than might be expected on the basis of geometry alone. However, DeMott⁷ found evidence that owing to the entopic scattering of light, the distribution of light energy on the retina does cover a larger area than previously thought possible.

SUMMARY

Differential thresholds for a series of small white light disks ranging from 0.6 to 20 minutes of arc diameter, as seen against an illuminated background of about 12 millilamberts luminance, were determined for foveal vision as the retinal image was blurred in fixed steps by being thrown out of focus.

The data show that the threshold is increased with increase in out-of-focus blurring of the retinal image, but this increase is proportionately less as the size of the disk is made larger. For very large sizes the threshold was more nearly independent of the blurring of the image.

The data showed that for disk sizes smaller than about three to four minutes of arc, the effective retinal-image size approaches a minimal limit of about six minutes of arc. Also, the differential threshold obeys Ricco's law asymptotically with disk sizes smaller than a critical angle of about five minutes of arc, and it approaches constancy with larger sizes. Again, with a given degree of blurring, Ricco's law is adhered to though there is an increase in the size of the critical disk.

These results imply that the size of the minimal effective retinal image is determined more by dioptric factors than by quasi-independent retinal areas within which summation of luminous energy is assumed to occur.

Mayo Clinic and Mayo Foundation.

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VERGENCE, AND ACCOMMODATION

♦ V. PUPIL SIZE CHANGES ASSOCIATED WITH CHANGES IN ACCOMMODATIVE VERGENCE.*

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INTRODUCTION

The miosis associated with viewing near objects is a familiar phenomenon. Despite this fact, until relatively recently all of the descriptions of the relationships involved were subject to considerable dispute. Infra-red pupil photographs,¹⁻³ and subjective measurement⁴ have now shown, however, that pupil size decreases in a rather marked way as the accommodation (and the associated accommodative vergence) increase. On the other hand, only very slight changes in pupil size can be detected with changes in fusional vergence and even these slight changes occur in only about 50 percent of the subjects studied so far.[†]

These previous studies have been confined to a rather limited range of accommodation stimuli. For this reason it has not been possible to specify the relation between pupil size and accommodation at the maximum level of accommodation. Such information may be of considerable value in helping to understand the relation between innervation

to the ciliary body and changes in refraction of the eye.⁵ Thus the present study was designed to examine the relation between pupil size and accommodation (and accommodative vergence), particularly at the higher levels of accommodation.

METHOD

Measurements of pupil size, accommodation and accommodative vergence were made on three adult prepresbyopic observers as the accommodation stimulus was varied through a wide range. The apparatus is illustrated schematically in Figure 1. The subject was seated in front of a mirror haploscope which was carefully aligned so that each arm rotated about a vertical axis which passed through the center of rotation of the relevant eye. The half-silvered mirror (M_3), mounted on the left arm of the haploscope, was covered on its back surface by black cardboard. In this way the left eye saw only a small point of light reflected by the first surface of the mirror and this appeared slightly above the fixated row of letters seen by the right eye. This spot of light could be moved horizontally back and forth across the target face by rotation of the left haploscope arm about its vertical axis. The position of the arm could be determined from the convergence

* Assisted by a grant from United States Public Health Service B1578 C (2).

† A summary of the various controversies relating to this problem is beyond the scope of the present paper. The interested reader can find detailed discussions of these matters elsewhere.¹⁻⁴

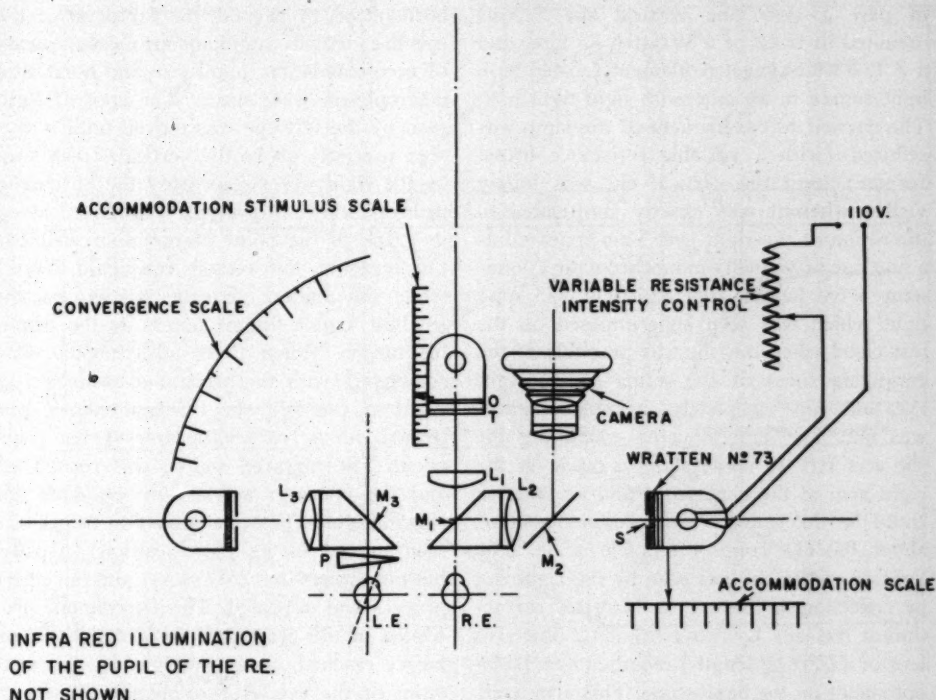


Fig. 1 (Alpern, Mason and Jardinico). Schematic drawing of the experimental arrangement as viewed from above. The power supplies for all of the electrical connections were stabilized by a suitable voltage regulation device, not shown in the figure.

(i.e., horizontal heterophoria) scale for this arm. An ophthalmic prism (15Δ or 20Δ) was placed base out in front of the left eye so that as the convergence increased to high values the apparatus did not interfere with the subject's nose.

A photographically reduced Snellen chart, consisting of black letters on a transparent background, was illuminated from behind an opal glass and a neutral density filter by a small $7\frac{1}{2}$ watt tungsten source connected to the 110 v. line. The target, opal glass, filter and light source were all mounted as a unit and were free to move back and forth along an optical track before the lens L_1 . This lens was a 122 mm convex achromatic objective lens. It was mounted before the right eye in such a way that the target was seen by this eye in a Badal optometer ar-

rangement by light transmitted through the half-silvered mirror M_1 . Hence, the accommodation stimulus could be changed continuously from $-8D$ to $+8D$ without variation in its size or luminance.

The chart luminance, as viewed through the instrument was 0.922 cd/m^2 . Since it was held constant throughout the experiment, the retinal illuminance varied directly with pupillary area. The extent of this variation was small ($0.428\log_{10}$ trolands for R.I.G.; $0.439\log_{10}$ trolands for M.A.; and, $0.452\log_{10}$ trolands for G.M.) and may safely be neglected in the present case since at this level of illuminance (about $1.0\log_{10}$ trolands) changes of this magnitude have only an extremely slight effect (of the order of 0.1 to 0.2 mm at the most) on pupil size.

To measure the amount of accommodation

in play a very fine vertical slit S was mounted in front of a Wratten 73 filter and a $7\frac{1}{2}$ watt tungsten filament frosted bulb light source in an otherwise light-tight box. The current to the filament of the lamp was reduced (with a variable resistance transformer) until the vertical slit was barely visible when it was exactly conjugated to the retina of the right eye. This appeared as a fine line of virtually monochromatic (dominant wave length approximately 575 m μ) light which was seen superimposed on the test chart when the slit was precisely at the conjugate focus of the retina of the right eye and was completely invisible when it was not. The lamp housing containing the slit was free to move along a track on the right arm of the haploscope and its position could be determined with the precision of about 0.025D from a suitable scale. The light from the slit was seen by the right eye by reflection at the mirror M_1 after refraction at the lens L_2 (an achromatic objective lens of 122 focal length) mounted as a Badal optometer on the haploscope. This arm itself was held in a fixed position with the angle of incidence of the line of sight of the right eye on the mirror M_1 equal to 45° . Because of this arrangement, light reflected from the iris of the right eye emerged from the lens L_2 , after reflection at the half-silvered mirror M_1 , essentially parallel. Thus an image of the iris could be easily photographed by reflected light from the half-silvered mirror M_2 . To illuminate the anterior segment of this eye a G.E. tungsten filament 6 v., 2.5 amp. lamp was mounted before a Wratten 89B infrared filter. The lamp and housing (not shown in Figure 1) were mounted above the line of sight and illuminated the eye at a distance of 8" in such a way that the line connecting the filament and the pupil of the right eye made a vertical angle of 45° with the line of sight. High speed infrared film (Kodak HIR-4-21) was used in the 35 mm camera.

In any given experimental session the stimulus target was placed in the neigh-

borhood of, or beyond, the far point of the eye and virtually simultaneous measurements of accommodation, pupil size and horizontal heterophoria were made. The spot of light seen by the left eye was moved until it was seen precisely above the vertical streak seen by the right eye (to measure the horizontal heterophoria); this streak was moved along its track to the point of optimum visibility (to measure the retinal conjugate focus) while the subject actively focused on the smallest legible line of letters on the stimulus target. When these adjustments were completed, with the accommodation held as fixed as possible, the infrared source was turned on and a photograph of the pupil taken. The infrared source was turned off and the observer relaxed in the dark for two minutes. The accommodation target was then moved to its new position (usually, but not always in 0.25D steps) and the entire process was repeated. The experiment proceeded in this way until the accommodation target reached, then came inside, the near point of the eye. Approximately 30 sets of measurements were made in each experimental run. For two observers eight repetitions of the experiment were made, for the third (R.I.G.), only a single experimental session was completed.

RESULTS

The results of these experiments are presented in Figure 2, which shows the mean data and illustrates the relations between: (a) accommodation and pupil size; (b) accommodation and accommodative vergence and (c) accommodative vergence and pupil size, for each observer.

The relation between vergence and accommodation for these observers is linear over a wide range. However, as the near point of accommodation is approached, vergence continues to increase even though accommodation levels off. The most reasonable interpretation is that this vergence is associated with innervation to the ciliary muscle which is not effective in variation in the re-

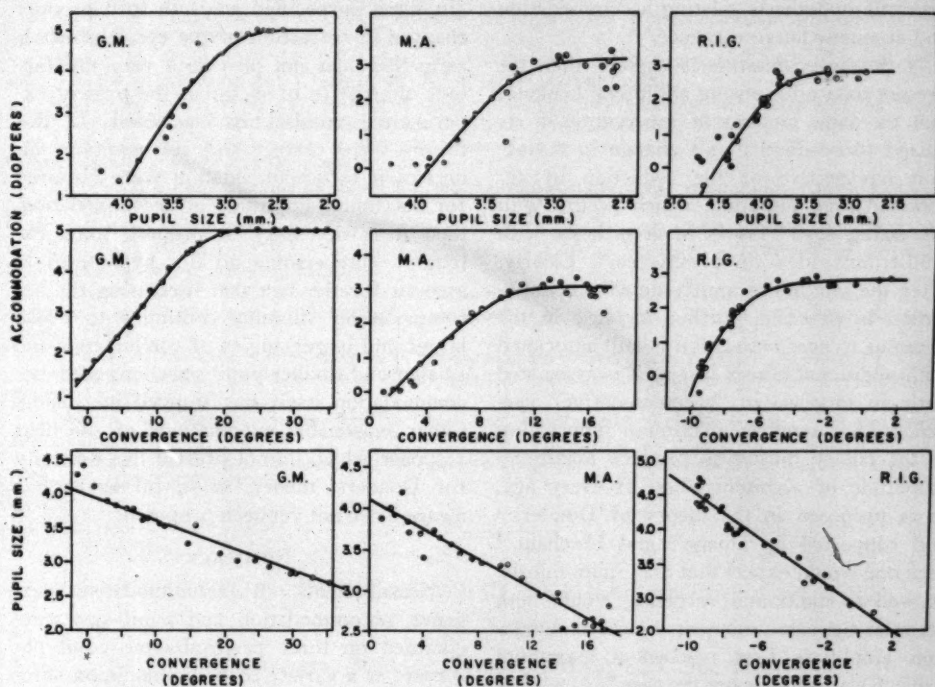


Fig. 2 (Alpern, Mason and Jardinico). Mean results of these experiments on each of the three observers. The results for R.I.G. are from a single experimental session; those for the other two observers are the means of eight repetitions in each case.

fraction of the eye because the lens has achieved the limit of its ability to vary its focus.⁶

The present results also demonstrate that pupil size is linearly related to change in accommodation over a wide range. However, as the near point of accommodation is approached the pupil size continues to decrease even after the accommodation reaches a maximum value. The most logical interpretation of this result is that the pupil size change is associated with an increased innervation to the ciliary muscle which is not associated with any further change of dioptric power of the eye because of the limitations imposed by the lens.

In support of this conclusion the bottom set of graphs in Figure 2 shows the relation between pupil size change and accommodation vergence. Within the limits of experi-

mental error this relation is reasonably approximated by a straight line.

DISCUSSION

Increased refraction of each eye, convergence, and miosis have been shown to occur concomitantly following unilateral faradic stimulation of a small region in area 19 of the occipital cortex of *Macaca mulatta*.^{7,8} It seems quite reasonable to propose that an increase in the accommodation stimulus of the eye results in physiological excitation of an analogous region in man, and that this provides the physiological basis for the synkinesis of the components of the near response. A considerable amount of evidence suggests that the innervations to each of the three components of the near response as a result of such excitation are linearly related to one another.⁶ This is an extension of a

previous hypothesis relating accommodation and accommodative vergence.^{6,9}

If this interpretation is correct, then the present data offer strong additional evidence that the same amount of innervation is required to produce a unit change in refraction irrespective of the reduction of accommodation amplitude which occurs with increasing age. This is in accordance with Gullstrand's idea of presbyopia.¹⁰ Clearly, after the maximum amplitude of accommodation is achieved, further increase in the stimulus to accommodation is still associated with additional miosis just as it is associated with an increase in "accommodative" vergence. If it required maximum innervation to the ciliary muscle to produce maximum amplitude of accommodation at every age, as is proposed in the theory of Donders¹¹ and supported by Duane¹² and Fincham,¹³ then one would expect that maximum miosis, as well as maximum vergence, would both occur at the same moment that accommodation amplitude first reached a maximum value. Clearly this is not the case.*

Adherents of the Donders' idea might be able to avoid this problem by some other theoretical interpretation of the relationships of the components of the near response. Although the theory of the near response just described seems the most reasonable explanation of the available data, the matter can by no means be regarded as settled. Fincham and Walton¹⁸ proposed that changes in the accommodation stimulus evoke vergence movements and that these latter

(in some unspecified way) in turn produce changes in refraction of the eye. Unfortunately this does not provide a very satisfactory alternative in so far as the present experimental results are concerned. If this theory were correct and if maximum innervation to accommodation were required for maximum amplitude of accommodation, then it is necessary to propose some extremely cumbersome ad hoc hypotheses to account for the fact that increasing the accommodation stimulus continues to evoke larger and larger angles of convergence and smaller and smaller pupil sizes long after accommodation itself has stopped increasing. Other reasonable explanations of the near response which do not present this difficulty for Donders' theory, in so far as we are aware, have not yet been proposed.

SUMMARY

Measurements of accommodative vergence, accommodation and pupil size were obtained on three prepresbyopic adult observers at a variety of accommodation stimulus levels. The results in each case showed a linear relation between accommodative vergence and pupil size, but a curvilinear relation between accommodation and accommodative vergence and between accommodation and pupil size. It seems most likely that these deviations from linearity are to be explained by the fact that in the adult observer the limitations on the amplitude of accommodation are imposed by the lens and that the innervation to the ciliary body can continue to increase even after the refraction change is maximum. This is evidence in support of Gullstrand's theory of presbyopia.

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* An analysis of the relative merits of these two conflicting views of presbyopia has been made by one of us elsewhere.¹⁴

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RECEPTOR ORIENTATION IN RETINAL PATHOLOGY

A FIRST STUDY*

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INTRODUCTION

In this paper an attempt was made to describe the behavior of the orientation of the retinal receptors in some pathologic conditions. This information was obtained from data relating to the directional sensitivity of the retina in the cases described. In addition, other tests allowing further evaluation of retinal function were employed in order to allow correlation of certain anatomical and functional factors present in a given case. Conditions were chosen where mechanical forces, acting either tangential or perpendicular to the retina may have been present in the pathogenesis of the disease, or may have

occurred in the course of the operational therapy.

The main category of pathology considered was retinal detachment. It can be assumed that in retinal detachment traction forces represent pathogenic factors, and that these same forces also affect those parts of the retina where, as yet, no separation has taken place. It may be further assumed that corrective therapy in detachment may initiate, neutralize, or supplement such forces. It is the ultimate goal of this series of studies to develop a pathogenic grouping (from a functional point of view) of retinal detachment, and if possible to determine means of early detection of some conditions leading to retinal detachment.

Other conditions considered were retinosis without detachment, peripheral retinal degeneration with hole formation, angiomatosis retinae, and retinal edema (as a consequence of disease or of therapy). Of particular interest were the effects of photo-

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coagulation of the retina and buckling procedures.

FACTORS MAINTAINING RECEPTOR ORIENTATION

As Zimmerman¹ has recently shown, the outer segments of the receptors are embedded in a ground substance chemically related to the so-called acid mucopolysaccharides. This ground substance might not only fulfill important metabolic functions, but also would act to maintain receptor separation which in itself is important.² It might also help to stabilize the orientation of the receptors, because of its inherent viscosity.

Microfibrils of the pigment epithelium are known to interdigitate with the outer members of the retinal receptors.³ These also aid in keeping the retinal receptors apart, and may play a significant role in receptor orientation.

MEASURES APPLIED

1. Direction of maximum sensitivity of the retinal receptors was determined. This measure provides information regarding the nature of the orientation of the central cones (as applied here).⁴

2. Stability in orientation of the retinal receptors (item 1 repeated at different times).

3. Sensitivity of the retinal receptors to light.

4. Resolution capability of the central retinal receptors.

Items 1-3 were determined by using a Stiles-Crawford apparatus.^{4,5} Item 3 was also studied by means of quantitative perimetric methods, and item 4 was determined by routine measurement of acuity using a grating target.

FORCES APPLIED TO THE RETINA

All forces applied to the retina and/or choroid may be resolved into tangential and perpendicular components. Tangential, employed in this sense, means that force was

applied parallel to the retina. In the normal patient, those factors which exert force upon the retina and choroid are intra-ocular pressure, rotation of the eye, accommodation, and "G" (gravitational) force.

In disease, in addition to variations in some of these factors, one has to deal with the effects of cicatrization, vitreo-retinal adhesions, and abnormal distributions of fluid. Softening effects and distortion resulting from buckling procedures, as well as traumatic events must also be considered.

TANGENTIAL FORCE

If a tangential force is applied to the normal choroid and/or retina, and differences exists in the elasticities of Bruch's membrane and the pigment epithelium, versus the external limiting membrane and the layer of rods and cones, it may be anticipated that the interdigitating fibrils will induce a disturbance in receptor orientation (simple tilt^{4,6}) if such forces are not applied equally in all radial directions relative to the retinal area in question. In the presence of force acting on both outer segments and fibrils, the interdigitations may be expected to dampen the effects of inertia. Further, one can imagine that a change in the physical properties of the ground substance or the fibrils could lead to increased instability in orientation of the outer segments of the receptors. This may contribute to the pathogenesis of retinal detachment. Once a microscopic separation of the fibrils and the outer segments of the receptors has occurred, or a partial to complete degeneration of the fibrils has taken place, and the ground substance becomes less viscous, local disturbances in orientation would probably not take place in the presence of a shearing force. In this instance, disturbances due to inertial response will probably occur more readily. It is difficult, however, to assess the effects of such occurrences upon visual response in as much as there would also probably be manifest disturbances in visual cell metabolism and

greater instance of optical interaction.² In such a case, one might expect less stability in time in the orientation of the visual receptors.

At this point it is of interest to note the fact that Zimmerman¹ has reported the disappearance of the ground substance in cases of retinal detachment. It is of importance to know whether this occurs prior to, or after detachment. If it occurs prior to detachment, and even if this substance is not essential to receptor metabolism, presumably more optical interaction² would take place and this would, in turn, give rise to small but real decrements in the resolution capability of the eye.

If the retina is exposed to a tangential stress having a vector component dominating in a given direction, we must consider possible changes in sensitivity in that direction due to the spreading of the grain.

PERPENDICULAR FORCE

Under ordinary conditions, the intra-ocular pressure tends to maintain the integrity of the layers of the eye. In this sense it acts to maintain normal receptor orientation, and to keep the visual cells in close approximation to their source of metabolites. This state exists only as long as no fluid becomes interposed between the pigment epithelium and layer of rods and cones.

Several forces exist which tend to separate the layer of rods and cones from the pigment epithelium by exerting a perpendicular force inward. Examples of such activity are "G" forces (which may be either tangential or perpendicular, and in the latter instance inward or outward) which might occur in the normal eye, and in disease, circumscribed edema or exudate, and vitreo-retinal adhesions. At the borders of such areas one must expect some tangential component and possible shearing. To the degree that separation occurs, varying degrees of disturbance in metabolism, and, hence, visual sensitivity may occur. To the degree that the ground

substance becomes less viscous one may anticipate more or less instability in orientation, and greater or less optical interaction and loss in resolution.

It is evident that at points of adhesion, i.e., the ora serrata, and at the optic nerve head, and at vitreo-retinal adhesions elsewhere, additional factors need to be considered. At the ora, the retina moves with the underlying choroid, e.g., in accommodation.⁷ At the disc, where both the retina and choroid are anchored, significant movement obviously does not occur. Gradients of force probably occur between these two limits.

In glaucoma, the effects of increased pressure upon the orientation of the retinal receptors are not yet evaluated.

Thus, in conclusion, the appearance of a retinal detachment would be influenced by the quality of the adhesion between receptors and pigment epithelium and magnitude and type of forces applied.

APPARATUS AND PROCEDURE

The Stiles-Crawford effect provides a convenient means of studying the orientation of the retinal receptors. These authors⁵ were the first to discover that the efficiency of light projected through different parts of the entrance pupil varies as a function of the distance of the test beam from the center of the entrance pupil of the eye.

Inasmuch as the retinal receptors exhibit directionality, one may assume that this directionality reflects the orientation of that which is directionally sensitive. Hence, in determining the direction of maximum sensitivity of the retina, one is able to study the oriented component. Enoch^{4,6,8} has studied the orientation of retinal receptors in amblyopia, and many of the concepts put forth in that work may be applied in retinal disease in general. Campbell and Gregory⁹ have very recently supported this work. In future studies, in cases of simple tilt, the directional visual acuity should be measured through the center of the pupil. This will

supplement and verify the work of Campbell and Gregory. Stiles¹⁰ has shown that the direction of maximum sensitivity varies in the same individual as a function of time. Ronchi¹¹ has presented data suggesting a change in pattern under the influence of mydriatics. Le Grand¹² has presented data showing a change in the direction of maximum sensitivity as a function of luminance.

The equipment used in his study for these measurements provided contrast threshold information as well as an evaluation of directional sensitivity in the central retina. As the apparatus employed is described at length elsewhere⁴ only a short description is given here. The light of a ribbon filament lamp was made monochromatic by means of a Farrand interference filter, having a half band width of 15m μ , and a peak wavelength of 556 m μ . The light of the lamp is divided by a beam splitter into two paths. These two beams each pass through two apertures. One of these pairs of apertures is conjugate with the plane of the entrance pupil of the eye, and one pair is conjugate with the retina. The display in the entrance pupil of the eye is duplicated in a monitoring device allowing exact positioning of the beams in the entrance pupil. A third light path is utilized in order to provide feedback information to the patient regarding his positioning relative to the instrument.

One beam formed an adapting field of 1.87° on the retina. This beam, of circular cross-section, had an area of 0.28 mm² in the entrance pupil of the eye, and was centered in the pupil. The test field subtended 0.58° at the retina and it could be moved about in the entrance pupil of the eye. The circular cross-section of this beam in the entrance pupil had an area of 0.06 mm.² Threshold determinations of the contrast threshold, or j.n.d., were obtained using an ascending method of limits (luminance increased in 0.02 log unit steps). These determinations were made at different points in the entrance pupil. The average duration of each exposure was 45.7 msec, and a pres-

entation was made every twenty seconds. Prior to the initiation of experimentation the patient was dark adapted and carefully positioned. Complete training sessions of three hours were held prior to test sessions. The pupil was initially dilated with 5 percent Euphthalmine and 10 percent Neo-Synephrine. Once the pupil had reached maximal size, further Euphthalmine drops were administered regularly in order to keep the pupil size constant in order to avoid a shift in the directional sensitivity curves (Ronchi¹¹). Some of the patients were already under atropine medication.

The visual acuity (in minutes of arc) was determined using a Bausch and Lomb Clason Acuity Meter. An artificial pupil of two millimeters diameter was employed. Screen luminance was approximately twenty foot-lamberts.

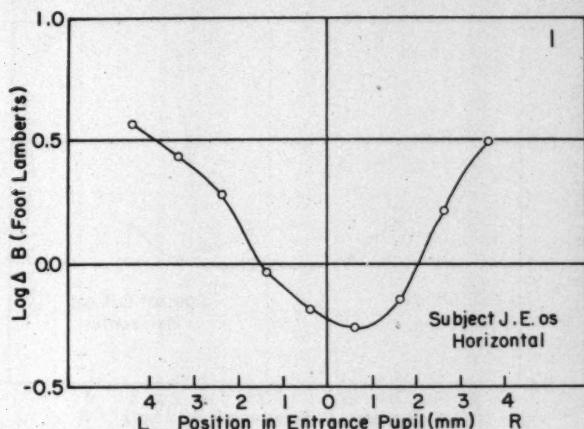
Kinetic and static quantitative perimetry were performed on a Goldmann perimeter equipped with the attachment for static quantitative perimetry. This apparatus has been described previously.^{13,14,15} The brightness of the cupola was set at 31.5 apostilbs (=2.93 ft.-L.).* The threshold values were obtained with a projection target, of 0.25 mm² area, having a maximum brightness of 1000.0 asb (=92.9 ft.-L.) which can be reduced in steps of 10 percent to 0.001125 asb. The patient was light adapted to the luminance level of the cupola prior to testing. Within 30° of the fixation point, the eyes were corrected with spectacle lenses. No correction was introduced for the lenses, or for the pupillary size, although these data are available.

RESULTS

Figure 1 shows a normal directional sensitivity curve taken in the horizontal meridian on experienced subject J.M.E. The ordinate is the contrast threshold expressed in log foot-lamberts, and the abscissa provides

* All charts and data regarding this instrument are ordinarily listed in apostilbs. Hence, we are using this unit.

Fig. 1 (Fankhauser, Enoch and Cibis). Normal directional sensitivity curve. Subject J.M.E.



the position of the test beam in the entrance pupil. Displacement of the test beam in the entrance pupil results in a change in the angle of incidence of the light striking the retina. These two factors, displacement, d , and angle of obliquity, Θ , may be related by the simple expression $\Theta = 2.5 d$. The direction of maximum sensitivity in Figure 1 is displaced slightly to the right. The normal limits of a deviation of the maximum of directional sensitivity from the center of the pupil are not well defined, but a first statistical estimate⁴ suggests a displacement of one millimeter in any direction as a limit.

Pathologic deviations from this normal curve can be classified as follows:^{4,8}

1. Simple tilt: A normal "U" shaped function displaced significantly from the center of the pupil. It is accompanied by a decrease in visual acuity, and a reduction in sensitivity. However, the sensitivity itself is probably minimally impaired, rather the ability of the eye to use the stimulus is reduced.

2. General Malorientation: A general flattening of the directional sensitivity curve, a reduction of visual acuity, and a reduction in sensitivity of the eye.

3. Reduced sensitivity without disturbance in orientation. This is a change in sensitivity of the eye independent of the orientation of the receptors.

CASE REPORTS

I. (C.R.) Retinoschisis O.D.

History: 30 year old male. The diagnosis of retinoschisis O.D. was established on routine examination. Refraction: $-1.00 - 0.25 \times 90^\circ$, V.A. = 1.0' separation. Fundus: Two cysts near ora at 8 o'clock. Fundus otherwise normal. Stiles-Crawford (directional sensitivity) curves prior to photocoagulation were somewhat flat (figs. 2, 3) but of "U" shaped form and the direction of maximum of sensitivity was almost perfectly centered in the entrance pupil. Directional sensitivity curves taken six days after photocoagulation (figs. 4, 5) show a strong tilt of the retinal receptors in both the horizontal and vertical meridians. V.A. = 1.6' separation. There was no reduction in maximum sensitivity of the eye.

Perimetric data reveals a steeper fall off in sensitivity on the nasal side of the field toward the periphery after photocoagulation.

II. (S.T.) Retinoschisis O.S.

History: 18 year old male. Rubber ball struck his left eye three weeks prior to admission. Refraction: $-0.50 \times 180^\circ$, V.A. = 1.9' separation. Fundus: Localized cyst near the ora between 2:30 and 3:15 o'clock. Fundus otherwise normal. A series of photocoagulations were performed. Directional sensitivity curves obtained prior to two days, seven days, and seventeen days after photocoagulation do not show any significant change in his direction of maximum sensitivity or in the shape of the directional sensitivity functions. The curve shows reasonable symmetry and absence of disorientation. However, it does show reduced maximum sensitivity to light of the order of one half log unit compared to the data in Figure 1. Data are not shown in the interests of economy of space. This can be classified as a case of reduced sensitivity with no change in orientation. This type case was predicted in the classification of amblyopia put forth by Enoch.^{4,8}

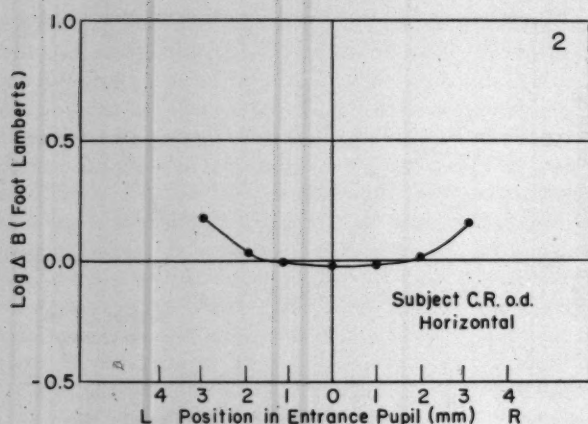


Fig. 2 (Fankhauser, Enoch and Cibis). Directional sensitivity curve (Stiles-Crawford curve) in a case of retinoschisis prior to photocoagulation. Case I.

Perimetric study reveals loss in sensitivity with indentation of peripheral isopters in the region of photocoagulation. As in the first case the loss in sensitivity is reflected quite some distance from the burn area in these determinations.

III. (C.R.) Retinoschisis with retinal detachment O.S.

Same patient as I. History: First symptoms one month prior to admission. Refraction: $-1.00 \times 180^\circ$ V.A. = 2.68' separation. Fundus: Retinoschisis forming a huge cyst in inferior half of the retina with retinal detachment occupying almost the entire lower half of the retina with sparing of the macula. The detachment of the retina was greatest in the periphery. At the macula there were degenerative spots which when viewed in optical section were located intra-retinally. Radial traction folds were visible in the macula region. Operation performed: encircling tube conducted subclerally between two

and seven o'clock, followed by two sessions of photocoagulations. Multiple burns were located in the lower temporal quadrant. The operation resulted in reattachment and considerable flattening of the cyst.

Stiles-Crawford curves before operation (figs. 6, 7) showed two possible maxima of orientation and a tendency toward general malorientation. Enoch has previously stated⁸ that general malorientation was probably the result of n groups of receptors oriented in different manners. These data suggest a small n in this case.

Postoperative: V.A. = 1.28' separation. The directional sensitivity curves (figs. 8, 9) showed greater regularity in orientation, but still some tendency for multiple groupings of oriented components. Sensitivity increased postoperatively. Most importantly, that which was inducing disorientation seems to have been decreased, and it seems that there is a process providing (at least in this in-

Fig. 3 (Fankhauser, Enoch and Cibis). Directional sensitivity curve in a case of retinoschisis prior to photocoagulation. The measurements were taken on a meridian passing through the center of the pupil. This is true for all successive determinations on the vertical meridian. Case I.

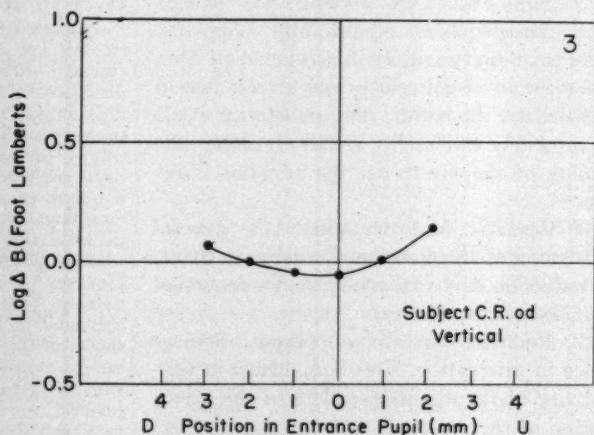
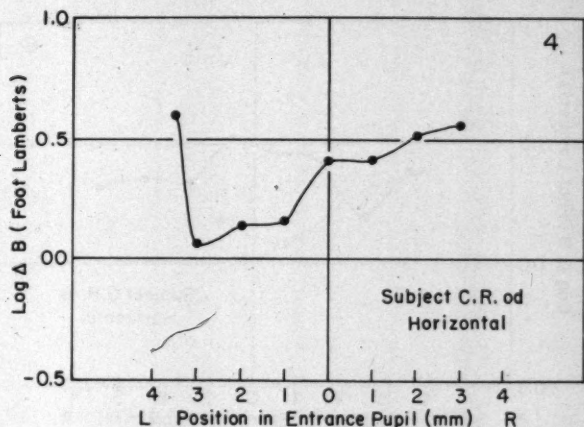


Fig. 4 (Fankhauser, Enoch and Cibis). Directional sensitivity curve in a case after photocoagulation. Case I.



stance) for recovery of proper receptor orientation and of sensitivity.

Perimetrically, prior to the operation, the kinetic method shows a loss of almost the entire superior half of the visual field, with exclusion of the macula (fig. 10). A similar result is registered with the static method. Loss of central and peripheral sensitivity is also noted (fig. 11). The increase of central and peripheral sensitivity after the buckling procedure is clearly visible (figs. 12, 13). Although both measures show an increase in field size and sensitivity the static method seems to provide a superior picture of the recovery process.

IV. (R.K.) Angiomas retinæ O.S.

History: 22 year old male. Two years ago a successful transscleral electrocoagulation was performed. Refraction: $-0.50 - 0.50 \times 180^\circ$, V.A. = 1.60' separation. Fundus: three large chorioretinal atrophic areas, located approximately as follows:

1., $\frac{1}{2}$ disc diameter below the macula, 2., approximately two disc diameters above the disc, 3., approximately 4 disc diameters temporally and superior to the macula. Traction folds were visible running over the macular area. The directional sensitivity curves show a simple tilt mainly in the horizontal meridian (figs. 14, 15). The sensitivity of the point of maximum sensitivity was essentially normal. The tilt must be considered as more or less fixed since it is still present two years after electrocoagulation. One would have expected a tilt in the vertical direction as there was a large atrophic area right below the macula. One has to remember however, that the resulting disorienting force must be the resultant of traction exerted by the three chorioretinal scars (situated as described above) and the disc.

Kinetic perimetry (fig. 16) shows a defect of the nasal superior quadrant. Slight indentation of isopters $\frac{1}{4}$ and $\frac{1}{3}$ inferiorly indicate the approximate orientation of areas of destruction.²

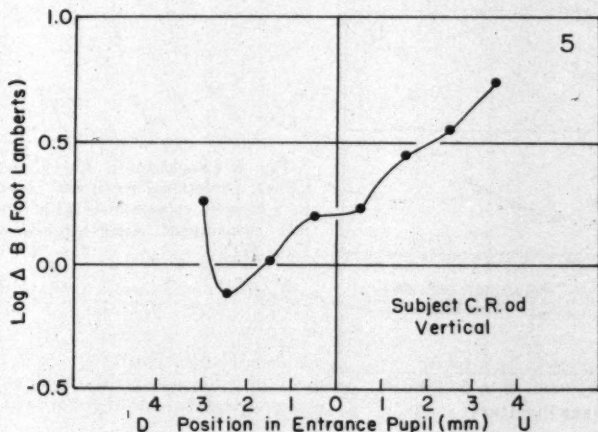


Fig. 5 (Fankhauser, Enoch and Cibis). Directional sensitivity curve in a case after photocoagulation. Case I.

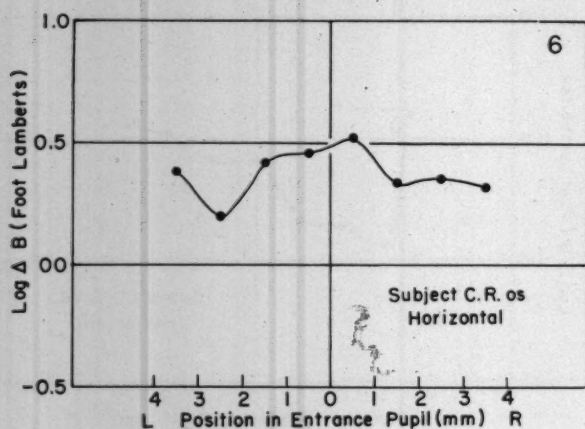


Fig. 6 (Fankhauser, Enoch and Cibis). Directional sensitivity curve in a case of retinoschisis with retinal detachment before operation. Case III.

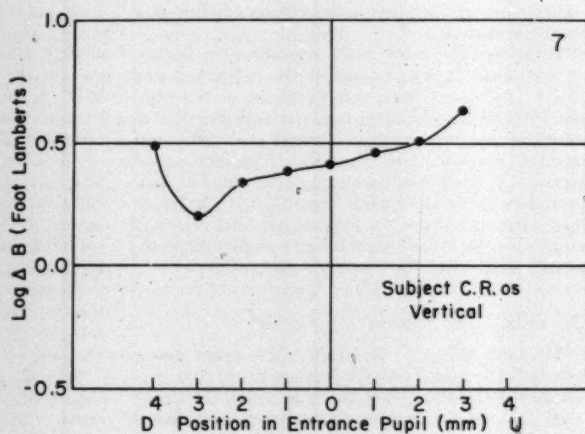


Fig. 7 (Fankhauser, Enoch and Cibis). Directional sensitivity curve in a case of retinoschisis with retinal detachment, before operation. Case III.

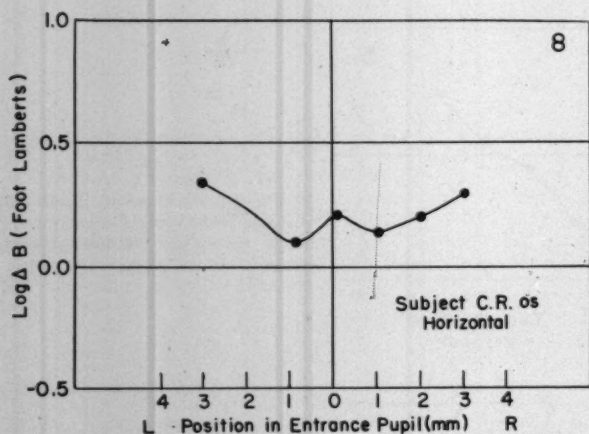
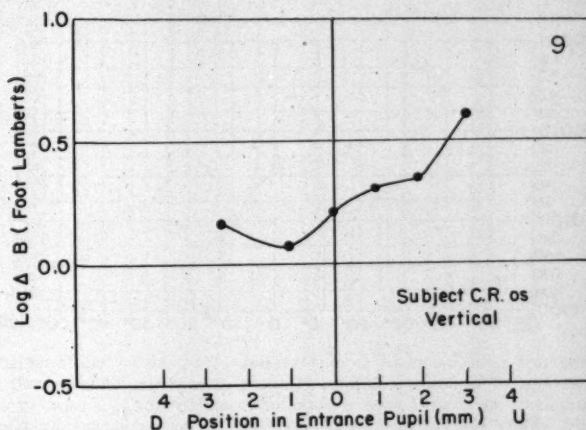


Fig. 8 (Fankhauser, Enoch and Cibis). Directional sensitivity curve in a case of retinoschisis with retinal detachment after operation. Case III.

Fig. 9 (Fankhauser, Enoch and Cibis). Directional sensitivity curve in a case of retinoschisis with retinal detachment after operation. Case III.



V. (R.K.) Angiomatosis retinae O.D.

Same patient as IV, however O.D. as yet not treated at the time of admission. Refraction: +1.00 V.A. = 3.22' separation. Fundus: Huge angioma of temporal superior retinal vessels, located three to four disc diameters superior and temporal to the macula. Semiclear edema of the retina at

posterior pole with fine white spots observed lying in the optical section in the swollen retina itself.

The directional sensitivity curves show almost complete disorientation and strongly reduced maximum sensitivity. One cannot compare these data with other data as the two curves presented (figs. 17, 18) have been taken with the exposure time of

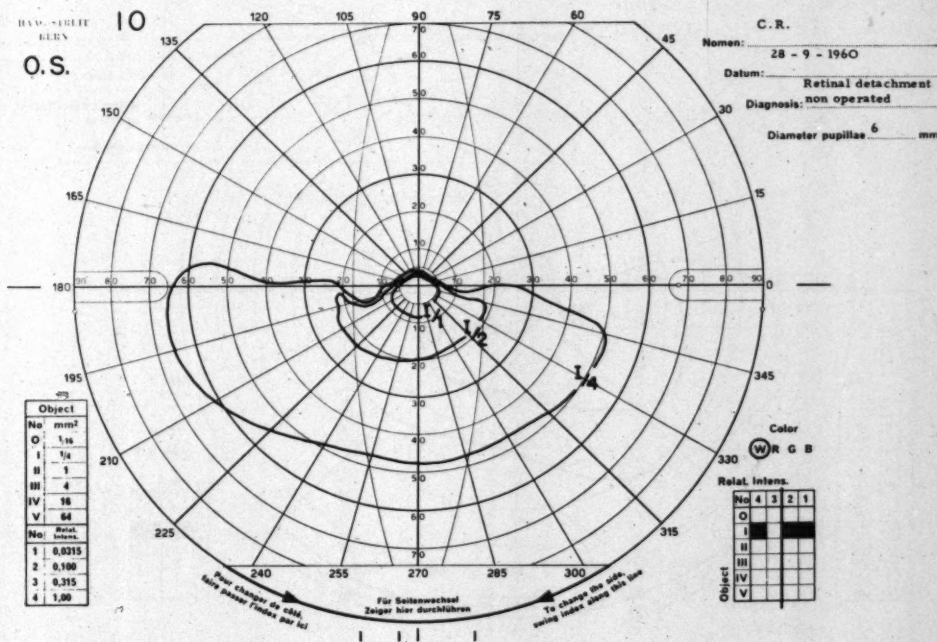


Fig. 10 (Fankhauser, Enoch and Cibis). Visual field determined by means of kinetic quantitative perimetry before operation in a case of retinoschisis with retinal detachment. Case III. Target size and relative brightness indicated on graph.

Fig. 13 (Fankhauser, Enoch and Cibis). Cross section through the visual field (meridian $0^{\circ}/180^{\circ}$) determined by means of static quantitative perimetric method in a case of retinoschisis with retinal detachment, after operation. See footnote under Fig. 11). Case III.

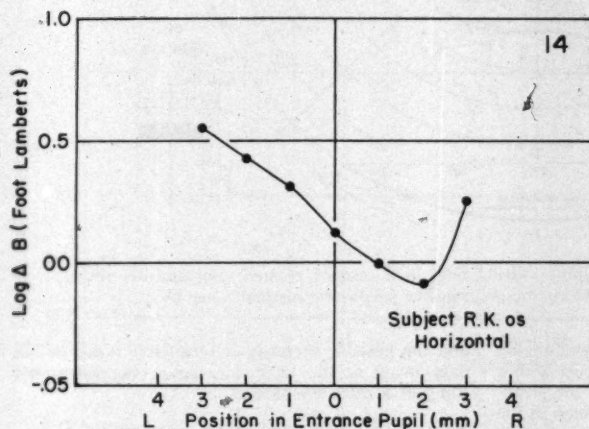
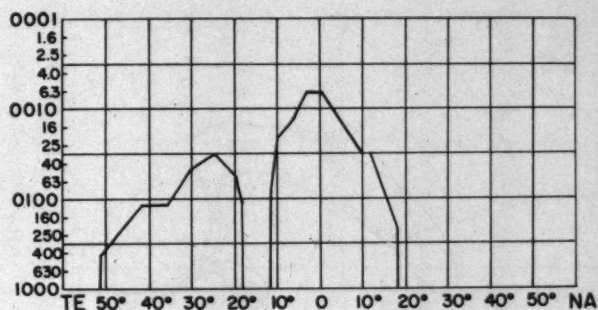
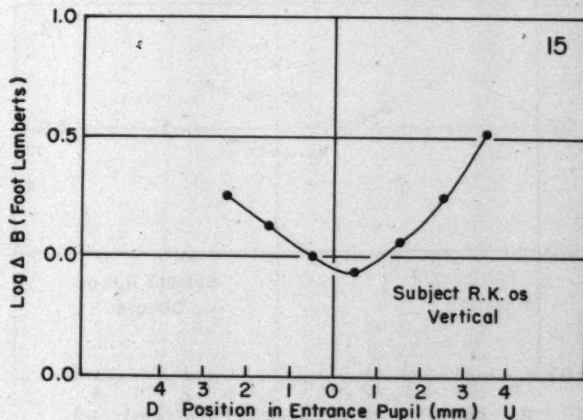


Fig. 14 (Fankhauser, Enoch and Cibis). Directional sensitivity curve in a case of treated angiomatosis retinae. Case IV.

Fig. 15 (Fankhauser, Enoch and Cibis). Directional sensitivity curve in a case of treated angiomatosis retinae. Case IV.



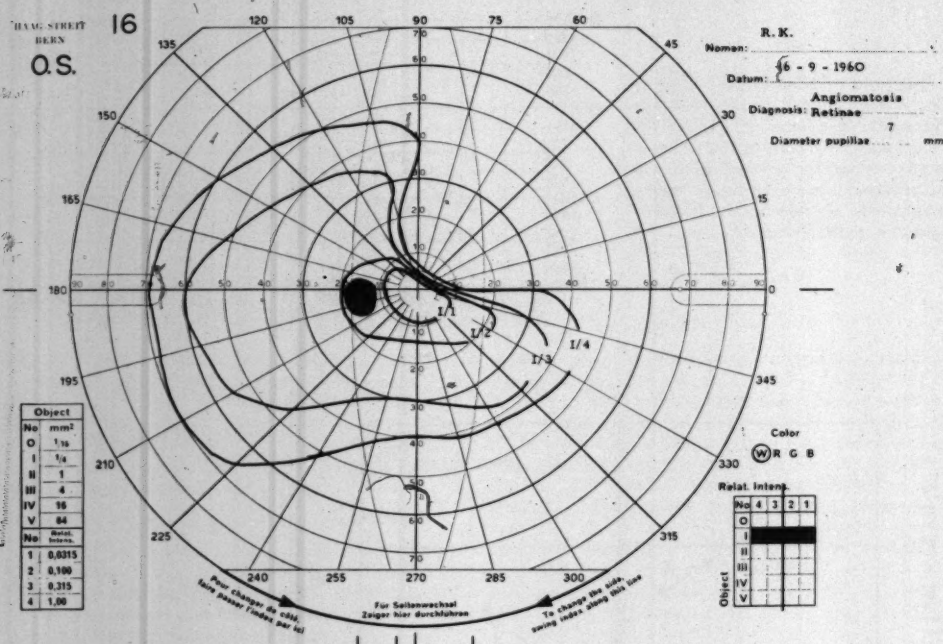


Fig. 16 (Fankhauser, Enoch and Cibis). Visual field in a case of treated angiomas retinae determined by means of the kinetic quantitative perimetric method. Case IV.

whitish areas (diathermy) in the equatorial region from six to one o'clock. Viewing the macula with a Hruby lens, a clear edema was visible at the posterior pole. Directional sensitivity curves taken in the horizontal meridian are presented in Figures 19 and 20. They exhibited general malorientation with reduced maximum sensitivity. The superior curve

and the possible increase in sensitivity noted in the data taken at the later date again suggest something of a recovery process.

VII. (M.S.) Idiopathic retinal degeneration O.S.

Same patient as VI. History: Discovered during routine ophthalmoscopy. Cystoid degeneration

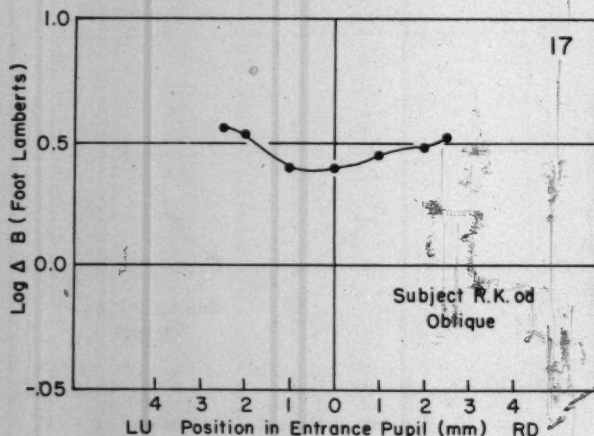


Fig. 17 (Fankhauser, Enoch and Cibis). Directional sensitivity curve determined in a case of angiomas retinae before photocoagulation, determined on meridian 45°/225° (no comparison permitted with other curves as exposure time had to be increased to 1 second). Case V.

Fig. 18 (Fankhauser, Enoch and Cibis). Directional sensitivity curve determined in a case of angiomas retinae before photocoagulation, determined on meridian $135^\circ/315^\circ$. Case V. (See footnote under Fig. 17.)

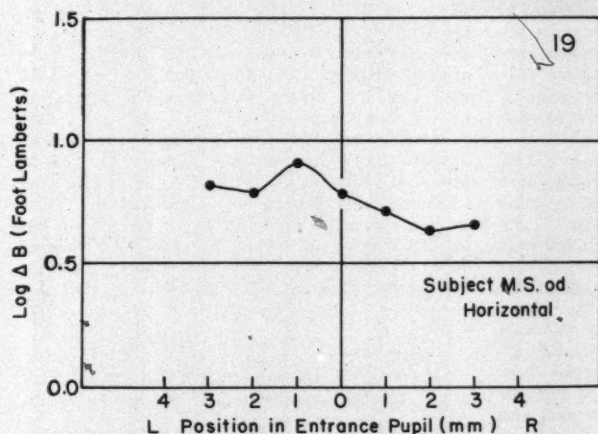
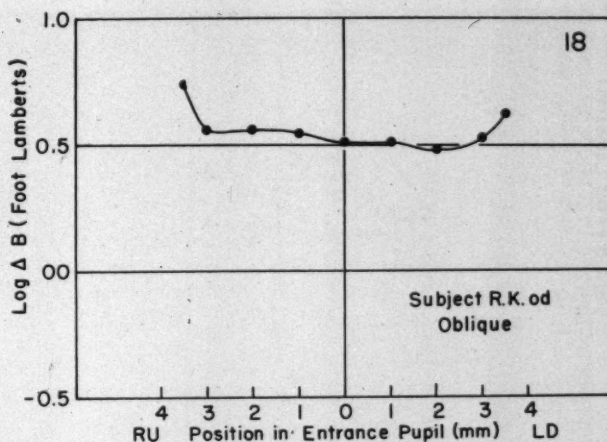
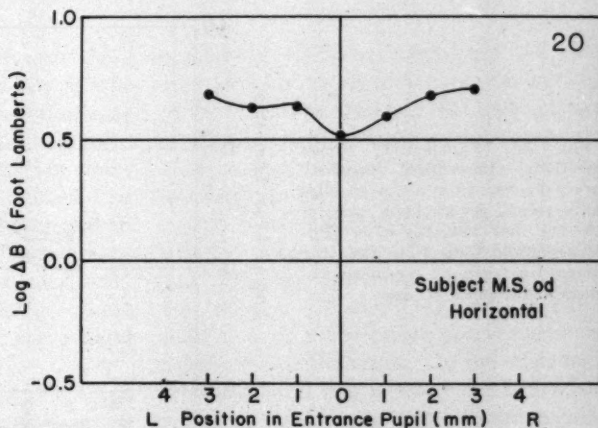


Fig. 19 (Fankhauser, Enoch and Cibis). Directional sensitivity curve determined in a case of idiopathic retinal detachment, 8 days after operation. (Note shift in ordinate). Case VI.

Fig. 20 (Fankhauser, Enoch and Cibis). Directional sensitivity curve determined in a case of idiopathic retinal detachment, 27 days after operation. Case VI.



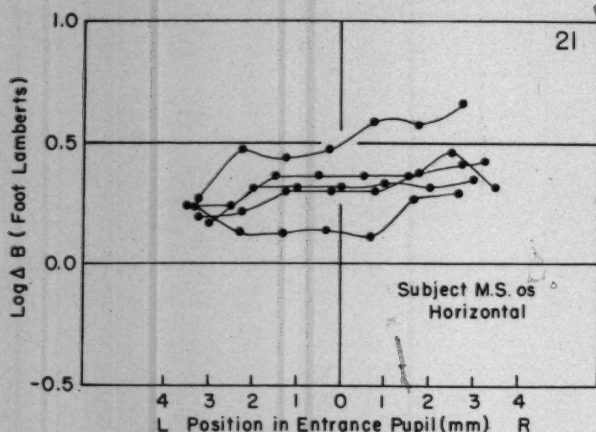


Fig. 21 (Fankhauser, Enoch and Cibis). Directional sensitivity curves determined in a case of idiopathic retinal degeneration taken at different time intervals after photocoagulation. Case VII. The top four curves are taken on the sixth and seventh postoperative day. The lowest curve is taken 23 days after photocoagulation.

with round holes and incomplete hole formations in the equatorial region were noted in the retina between 12:30 and 4:30 o'clock. Multiple photocoagulations were placed widely across the temporal half of the retina. No examination (from the point of view of this study) could be conducted prior to photocoagulation.

Postoperative: Refraction: -4.50 V.A. = $2.70'$ separation six days after photocoagulation, and V.A. = $1.65'$ separation 23 days after photocoagulation. Fundus: Multiple photocoagulation burns were visible across almost the entire temporal half of the retina in the equatorial region. In addition, residuals of the cystoid degeneration described above were seen. Directional sensitivity curves were taken twice each day at six hour intervals four and five days after operation (figs. 21, 22; ignore lowest curve in fig. 21). The curves show varying degrees of general malorientation and reduced maximum sensitivity in both horizontal and vertical meridians. The curves showing the lowest

sensitivity were the first of the series of four determinations. The lowest curve (highest sensitivity) in Figure 21 represents data taken 23 days after photocoagulation. These data show a marked improvement in sensitivity as is implied in the improved acuity data.

Although the variation in the series of four sets of data (top four curves in Figure 21, and all curves in Figure 22) may well be due to individual variation, it is tempting to suggest that the differences occurring in the first set of these four sets of data imply some change in orientation occurring quite rapidly in this eye. The change occurring during the following weeks in both eyes certainly lends some weight to this possibility.

DISCUSSION AND CONCLUSIONS

These data provide much in the way of interesting material. In general no new categories are added to the format presented by

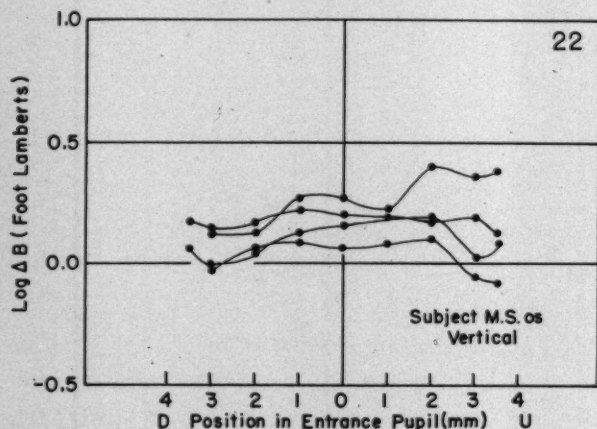


Fig. 22 (Fankhauser, Enoch and Cibis). Directional sensitivity curves determined in a case of idiopathic retinal degeneration taken at different time intervals after photocoagulation. Case VII. The four curves are taken on the sixth and seventh postoperative day.

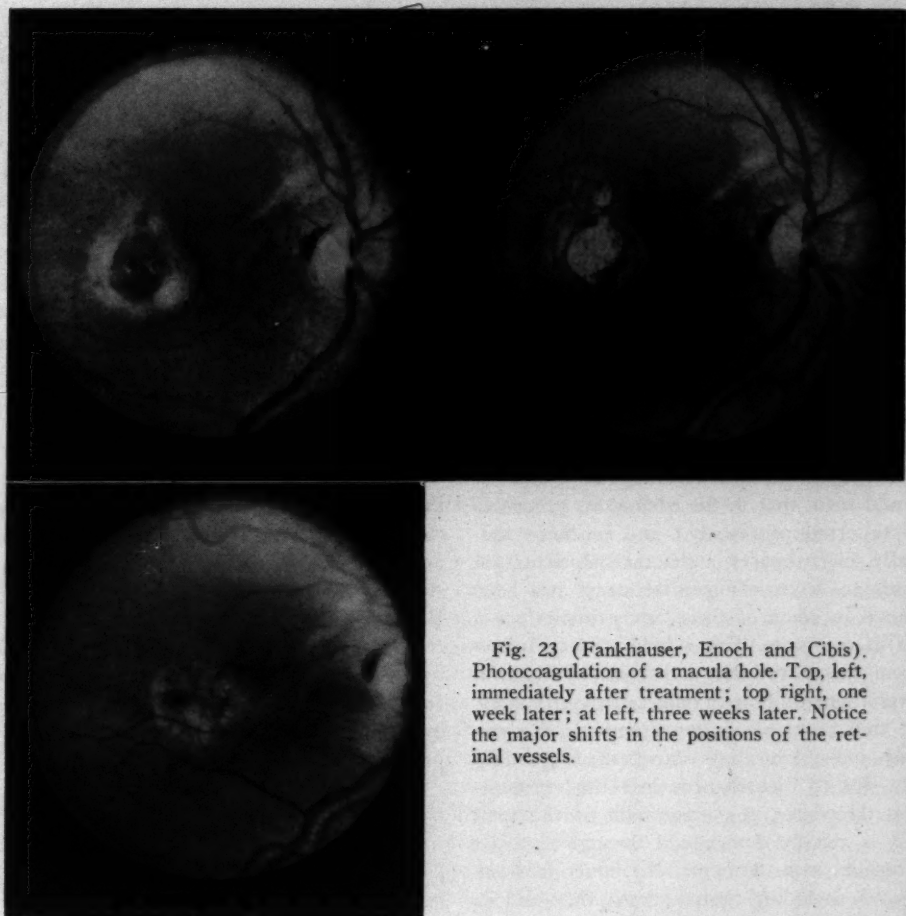


Fig. 23 (Fankhauser, Enoch and Cibis). Photocoagulation of a macula hole. Top, left, immediately after treatment; top right, one week later; at left, three weeks later. Notice the major shifts in the positions of the retinal vessels.

Enoch in the classification of Receptor Amblyopia.⁸ A pure case of loss of retinal sensitivity without tilt or malorientation was found. Since no prior normal data were available on this patient, no clear cut statement of the magnitude of the loss could be made. In addition, a general malorientation with a small number (n) of contributory components was found. This is interpreted as supporting the previously stated concept concerning the pathogenesis of the general malorientation category.

If one can induce simple tilt of the retinal receptors (Case I) by photocoagulation, it is

conceivable that one might use the same method to correct disturbances in orientation of the same type. The fact that these changes occur after light coagulation represents still another proof that the Stiles-Crawford effect has origin in the retina.

It is of significance that in some individuals the stress imposed by photocoagulation and pathology apparently does not disturb the retina at a distance, and that in others there may be a mechanism providing for re-orientation of the retina. On the other hand it is significant that in many cases, resultant changes in sensitivity and orientation occur

at rather large distances from the site of photocoagulation. It is important to observe the marked differences in response of the individual retinæ to insult. This variability may be due in part to those factors related to location of the lesions, and the rate and magnitude of the post-procedural cicatrization (coagulation). However, it must be due in part to the stability of those factors which tend to maintain the integrity of the retina, and the magnitude of the forces applied tangentially and perpendicularly to the retina. At this time, it is difficult to attribute the disorientation of patterns we have found to any specific disorienting force which has been postulated above. However, some qualified general statements may be made. It would seem that in the edematous processes as reported previously,⁸ and in these specially selected cases, a general malorientation tends to occur. Tangential stress has been shown in some of these cases to produce a tilt in receptors. This would tend to follow from the introductory discussion. Loss in retinal sensitivity may reflect many processes. At this time, using these methods, we can only make causative statements regarding loss due to disturbances in retinal orientation. However, the magnitude of the total loss is readily determined through contrast threshold measurements. No doubt in some lesions, including retinal edema, there is loss

in sensitivity due to disturbance of the metabolic processes, etc. Where forces exist which have a perpendicular component which acts to separate the retina from its substrate, a metabolic component may exist.

It is felt to be wrong at this time to attempt to carry these arguments too far, in view of the complexity of these cases. To refine these statements, further experiments must be conducted allowing for quantitative evaluation of the effects of force applied to the retina. At this time, the method employed in this study promises to be useful from a prognostic and diagnostic point of view.

ADDENDUM

It is important to show the magnitude of the traction forces induced by chorio-retinal cicatrization. This is best illustrated in the accompanying figure (Figure 23). In this instance a nineteen year old male patient (W. B.) manifested a macula hole. He was treated by means of photocoagulation. The figure shows how, in the weeks immediately following treatment, the retina, as indicated by the position of the retinal vessels, was pulled toward the macula.

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A PLASMA-MANNITOL-BROM CRESOL PURPLE AGAR FOR RAPID IDENTIFICATION OF STAPHYLOCOCCI.

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"Prepare to meet thy God" was the ominous warning hanging over the entrance to the surgery ward in Aberdeen, Scotland, when Sir Alexander Ogston (1844-1929), an important contributor to the understanding of the role of Staphylococci in wound infections, was Professor of Surgery. However, after Sir Alexander visited Edinburgh and saw Lister's carbolic acid spray "method of avoiding suppuration and blood poisoning in operation wounds," he "saw that a miraculous change had come over our Science, and my mind was almost bewildered with the glorious visions of all that it entailed." Ogston returned to Aberdeen and shocked everyone by tearing down and burning the grim text.¹

Today, the Staphylococcus is still an important agent of disease, and one which can turn a successful eye operation into a catastrophe overnight. We can still heed the dour Scottish warning with the ever-present reminder that an uneventful operation may terminate in a disastrous postoperative infection. "Prepare to meet thy God" may not be a necessary part of preoperative care for eye surgery, but adequate consideration of

the role of Staphylococci certainly is, since these organisms are almost always the cause of a postoperative infection.²

Staphylococci are so common that it may seem to the clinician that a bacteriology laboratory can grow nothing else. Since these organisms are so ubiquitous, to the medical bacteriologist it is often important to determine rapidly whether a given organism is a pathogen or a harmless contaminant. Although this taxonomy may not be perfect,³ currently Staphylococci are classified into two groups for clinical purposes.⁴ The most important and most frequently pathogenic is the Staphylococcus aureus (Staphylococcus pyogenes), which produces the enzyme coagulase and ferments mannitol. The other is the Staphylococcus epidermidis (a more inclusive term than the older Staphylococcus albus), which does not produce coagulase or ferment mannitol, and rarely causes disease.

Differentiation of these two is of paramount clinical importance. Pigment production is of no value in distinguishing a potential pathogen from a non-pathogen since the usually golden Staphylococcus aureus may produce yellow, orange or no pigment at all, and non-pathogens may be pigmented.^{5,6} Neither is hemolysis, since there are three different hemolysins, alpha, beta and delta, produced by Staphylococcus aureus, all of which differ from the one or more hemolysins produced by Staphylococcus epider-

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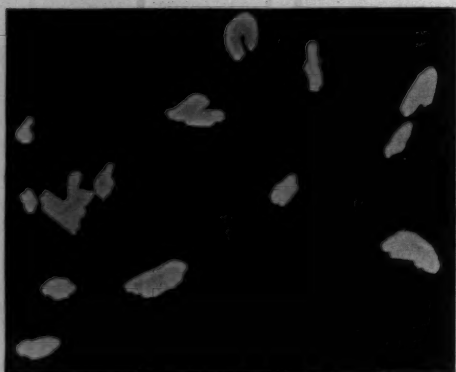


Fig. 1 (Burns and Florey). Plasma-Mannitol-Brom cresol purple agar. Blood agar plate showing large zones of hemolysis around colonies of *Staphylococci* photographed in transmitted light. Hemolysis is not a valid criterion of pathogenicity.

midis.⁷ The hemolytic pattern will vary with the source of the blood used in a blood agar plate, and a large zone of hemolysis may be seen with many strains of *Staphylococcus epidermidis* (fig. 1). However, production of alpha hemolysin, which lyses rabbit red blood cells, correlates quite closely with coagulase production and has been suggested as a criterion of pathogenicity.⁸

The most valid but least desirable way of determining the seriousness of the infection is by seeing how sick the organism makes its human host. Because of the impracticality of this approach, it is customary now to differentiate species of *Staphylococci* by the coagulase test,⁹ and many laboratories now report *Staphylococci* simply as coagulase positive or negative, instead of *aureus* or *epidermidis* (*albus*), and omit description of hemolysis.

An important consideration in ophthalmic bacteriology is time. Because of the rapidity of permanent loss of sight in eye infections, accurate speed in laboratory determinations is of greatest importance in conditions such as endophthalmitis or corneal ulcer. Suie¹⁰ has recommended thioglycollate, a liquid aerobic-anaerobic culture medium, for routine ophthalmologic use. This is a valuable medium, but a culture submitted to a labo-

ratory in thioglycollate would have to be grown out, the organisms isolated by streaking on a plate the next day, and a third day used for the *Staphylococcal* coagulase test. If a blood agar plate is used as a culture medium a second day may be necessary for coagulase determination.

The coagulase test is usually done by placing a fresh broth culture of *Staphylococci* into a tube of human plasma, diluted with saline to weaken coagulase inhibition by the plasma. Coagulation may occur in one to twenty-four hours, but the plasma clotted to fibrin in the first few hours can be liquified by twenty-four hours if the same organism also produces large quantities of fibrinolysin. Hence, repeated observations are necessary. This tube technic measures free coagulase liberated into the medium during bacterial growth. A second, quicker technic, that measures coagulase bound to the cell instead of the diffusible coagulase assayed by the tube coagulase test, is the slide test. The two methods do not correlate entirely. A third method, to be described here, utilizes the diffusability of free coagulase from the originally isolated bacterial colony into a solid medium containing plasma. If the culture medium surrounding the growing bacteria turns cloudy from precipitated fibrin, coagulase is assumed to be present.

Another valuable criterion of the pathogenicity of *Staphylococci* is the mannitol test, which was advocated by Thygeson.¹¹ This is valid because pathogenic *Staphylococci* ferment mannitol to an acid, thus changing color of a pH indicator incorporated in the medium, which is usually at a pH of 7.3 to 7.4. Phenol red, which turns from red to yellow if acid is present, is the most commonly used indicator dye.

An agar incorporating plasma, mannitol and brom-cresol purple (dibromo-o-cresol-sulfonphthalein) has been devised by Esber and Faulconer¹² and modified by us (fig. 2). In this agar, plasma is incorporated to determine diffusible coagulase and mannitol is added, which can be fermented to acid

changing the color of the indicator, brom-cresol purple, from deep blue to yellow. We have not found it necessary to adjust the pH of the medium after preparation and autoclaving.

If Staphylococci are plated out in the initial culture, as early as eight hours easily identifiable cloudy yellow areas around the pathogenic aureus colonies may be seen with the organisms producing a large amount of coagulase. The cloudy zone is best seen in transmitted light (fig. 3). This medium provides a rapid overnight test to pick the *Staphylococcus aureus* from the frequent non-pathogens. This, of course, still does not tell one which is the antibiotic of choice for treatment, and antibiotic sensitivity testing should be done as indicated. In an acute infection, such as a corneal ulcer, the residents of the Ophthalmology Department at the University of Oregon Medical School are trained to collect one set of plates for isolation of the etiologic agent and a second for determination of antibiotic disk sensitivity on the original material, since there is a 96 percent correlation of results with the two techniques.¹³

This agar plate method of coagulase and mannitol determination was checked for accuracy on 234 specimens of Staphylococci, obtained from the University of Oregon

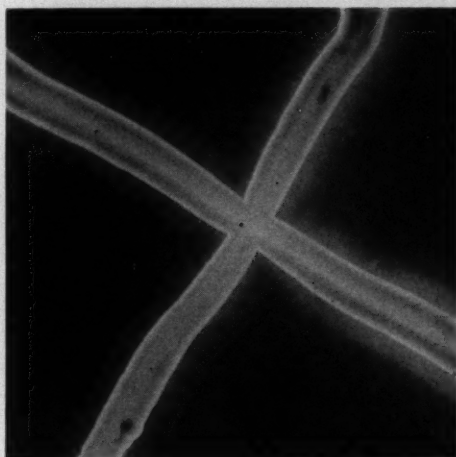


Fig. 3 (Burns and Florey). Plasma-Mannitol-Brom cresol purple agar. Cross-streaked culture of *Staphylococcus aureus* on plasma-mannitol-brom cresol purple agar, photographed in transmitted light. The hazy zone around the growth identifies the organisms as coagulase positive, and the cloudy zone of agar has turned from blue to yellow, indicating mannitol fermentation. Both are valid indicators of pathogenicity.

Medical School Clinical Laboratory and from the Microbiology Laboratory in the Department of Ophthalmology. Coagulase determinations done by the tube method, inoculating a loopful of a single colony into plasma diluted at least ten times with saline, read at four and twenty-four hours for clotting, were compared with the slide coagulase technic, done by mixing a loopful of a single colony into saline and adding a loopful of fresh plasma. A saline control was done on each slide coagulase test, and the results were read immediately for macroscopic clumping. Mannitol determinations were performed by overnight incubation on Difco mannitol-phenol red agar tubes, and the result was considered positive if the medium turned from red to yellow.

None of these methods seemed perfect. The agar plate coagulase agreed with the tube coagulase determination on 223 of 234 specimens (95 percent). In the eleven instances of disagreement there were seven

PLASMA - MANNITOL AGAR		
	Original	Modification
Brain-heart infusion broth	0.5 gm	
Brain-heart infusion agar		1.4 gm
Trypticase soy agar	2.8 gm	1.4 gm
Plain agar	0.8 gm	
Mannitol	1.0 gm	1.0 gm
Brom-cresol purple 1.6%	0.1 ml	0.1 ml
Distilled H ₂ O	100 cc	100 cc
7cc plasma added before pouring		

Fig. 2 (Burns and Florey). Plasma-Mannitol-Brom cresol purple agar.

where the plate method was positive and the tube negative, and four where the plate was negative and the tube positive. Correlating these eleven with the tube mannitol test gave seven in which the tube mannitol agreed with the tube coagulase and four in which the tube mannitol agreed with the plate coagulase. It was concluded that the plate and tube coagulase determinations were of about equal accuracy. The slide coagulase test was felt to be less accurate, since about one in ten was unreadable because of spontaneous clumping in saline.

In comparing the plasma-mannitol-brom cresol purple plate with tube mannitol-phenol red agar determinations, there was agreement in 214 of 234 (91 percent) specimens. In thirteen specimens the plate was positive and the tube negative, in seven the plate was negative and the tube positive. Correlating these twenty discrepant specimens with the tube coagulase test showed that in twelve the tube coagulase agreed with the plate mannitol and in eight the tube coagulase agreed with the tube mannitol. The plate method did not seem to be grossly less accurate than the tube mannitol test done on phenol red mannitol agar, and if anything would show fewer false negatives.

It was concluded that the plasma-mannitol-brom cresol purple agar is a clinically sufficiently accurate method for determination of coagulase production and mannitol fermentation.

This plasma-mannitol-brom cresol purple agar is useful for routine ophthalmic bacteriology, since the common organisms, including the most difficult to isolate, grow readily. This is an advantage over selective media which inhibit other organisms than *Staphylococci*, such as the high concentration sodium-chloride medium of Chapman¹⁴ and the tellurite-glycine agar of Zebovitz et al.¹⁵

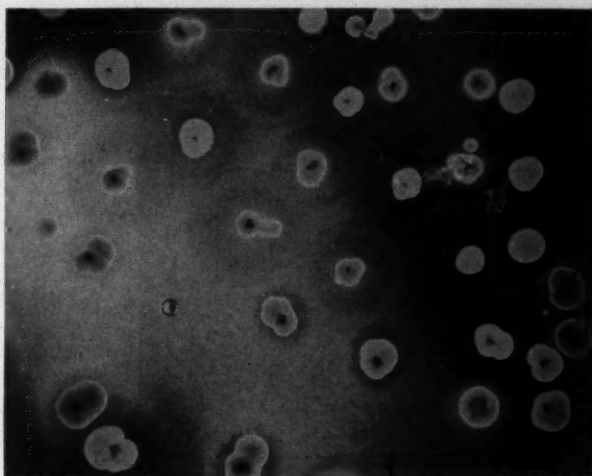
A cultural characteristic of *Hemophilus influenzae* and *Hemophilus aegypti* (Koch-Weeks bacilli) is the satellite phenomenon, or increased growth of the colonies adjacent

to colonies of *Staphylococci*, which can be seen on plasma-mannitol agar. *Moraxella lacunata* (*Morax-Axenfeld bacilli*) also grow well on this medium. *Pneumococci* show a slight green hemolysis on blood agar, and at times there may be a slight indication of mannitol fermentation on the plasma mannitol agar. *Streptococci* grow well on this agar, since it is enriched with plasma. *Gonococci* are unusual eye pathogens that are difficult to grow, but in three of five mixed specimens obtained from genital cultures from the City of Portland Laboratory growth was obtained on plasma-mannitol agar. In one a *Mima* was obtained and overgrowth of other organisms may have prevented isolation in the fifth. The usual gram negative organisms such as *Pseudomonas* and *Proteus* grow easily on this medium and the green pigment of the *pyocyaneus* may be visible. *Escherichia coli* are the organisms most likely to be confused with *Staphylococci* since they also are coagulase and mannitol positive. However, the gross colony characteristics are adequate for differentiation since the *Escherichia coli* colonies are larger, more mucoid, and usually show coagulase only after two to four days incubation.

Since January, 1960 at the University of Oregon Medical School Department of Ophthalmology preoperative cultures have been done by the resident staff on Sunday on the patients to be seen at preoperative conference on Monday. Both conjunctivae and both anterior nares are cultured, and streaked on both blood agar and plasma-mannitol agar plates for comparison of the twenty-four hour results.

It has become apparent that, after overnight incubation, the plasma mannitol agar is a more rapid way of identification of *Staphylococcus aureus*, since no transfer to other media for other tests is necessary. A second advantage is that identification of small numbers of *Staphylococcus aureus* in a mixed culture is more easily accomplished than with blood agar, since the blue-yellow change is much more easily discernible than

Fig. 4 (Burns and Florey). Plasma-Mannitol-Brom cresol purple agar. Colonies of coagulase positive and negative *Staphylococci* on plasma-mannitol-brom cresol purple agar, photographed in transmitted light. The white halo around most colonies is fibrin, and some colonies exhibit a halo of clearing due to fibrinolysin.



yellow pigment production. However, for optimal results, this medium should be used in conjunction with blood agar plates and thioglycollate tubes.

There are minor drawbacks to this medium, of which the most important is the plasma. We use outdated citrated blood bank plasma. Different lots of plasma may vary in ability to show coagulase activity of *Staphylococci* partly because of anticoagulase activity of the plasma. A plasma suitable for slide coagulase tests may be unfit for tube or plate tests. Pooling of separate lots of plasma is advisable, and a good batch may be kept frozen indefinitely before use. Each new plasma should be checked against known strains of *Staphylococcus aureus* of each of the four bacteriophage groups, since each of the four groups possess an antigenically different coagulase. Most lots of plasma are usable.

Secondly, many *Staphylococci* produce fibrinolysin which can cause a zone of clearing inside the cloudy fibrin zone (fig. 4). However, in contrast to the rapid liquefaction of the clot in a tube coagulase test, where a test that is positive at four hours may be negative in twenty-four, thus requiring two or more readings, with the plasma-mannitol agar there is no clearing present

until twenty-four to forty-eight hours. A false negative will not appear until three to four days, when the entire plate may lose the cloudy appearance of coagulated fibrin. Others have found beta toxin or other substances to produce an opacity simulating that associated with coagulase, and for these and other reasons have described the plate method, using other mediums, as unsatisfactory.³

A less frequently noted objection is the degree of pH shift necessary for color change of the indicator. The plasma-mannitol agar requires more acid to change color than the mannitol agars currently in use, because of the increased buffering action of the plasma-supplemented agar and the lower pK of the brom-cresol purple. If the colony does not ferment enough mannitol to change the color of the medium the colony itself will be bright yellow even though the surrounding medium is unchanged. The bright contrast between the color change from blue to yellow, compared to the less noticeable color change from red to yellow with regular mannitol agar, is an advantage, as is the fact that brom-cresol purple breaks down and changes color less readily than phenol red if autoclaved a longer period than necessary.

The only other known source of confusion

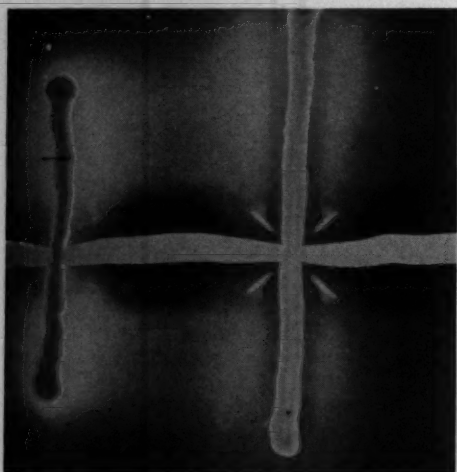


Fig. 5 (Burns and Florey). Plasma-Mannitol-Brom cresol purple agar. Cross-streaking of coagulase positive (vertical) and coagulase negative (horizontal) Staphylococci on plasma-mannitol-brom cresol purple agar, photographed in transmitted light. Four precipitin bands appear at juncture of growth of one strain of coagulase and fibrinolysin positive Staphylococcus with the coagulase negative Staphylococcus.

arises out of the fact that some strains of *Staphylococcus epidermidis* produce a substance of unknown nature, which causes the colony to be surrounded by a narrow sharply

defined opaque area, in contrast to the diffuse coagulum produced by *Staphylococcus aureus*. If the plasma mannitol agar plates are allowed to stand at room temperature for several days precipitin bands are sometimes formed between coagulase positive and negative colonies (fig. 5).

SUMMARY

1) Rapidity and accuracy of identification of pathogenic bacteria is an important consideration for maximal therapeutic results in ocular infections, particularly in serious conditions such as corneal ulcer or endophthalmitis.

2) A plasma-mannitol-brom cresol purple agar is described as a solid medium which has a broad usefulness for general bacteriologic work and a particular importance from the ophthalmologic standpoint, since in as little as eight hours coagulase and mannitol positive Staphylococci can be identified.

University of Oregon Medical School.

ADDENDUM

Since submission of this manuscript, this medium has become commercially available through Baltimore Biological Laboratory, Inc.

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THE COEFFICIENT OF SCLERAL RIGIDITY. EFFECT OF VARIATION OF THE INTRAOCULAR VOLUME*

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INTRODUCTION

In a recent series of experiments by Phillips and Quick on hollow, water-filled rubber spheres, it has again been shown that a given change in volume, produced by indentation or other methods, will result in a change in pressure which is an inverse function of the initial volume of the sphere.¹ This appears to be analogous to the situation in which eyes larger than those on which an indentation tonometer was calibrated will give an apparently low reading of intraocular tension, whereas the tension will be misleadingly high with smaller eyes.²

Although the influence of the initial volume on pressure-volume increments has been known for some time,^{2,3} the most widely used relationship between pressure and volume changes within an eye omits consideration of it entirely:

$$K = \log_{10} \left(\frac{P_2}{P_1} \right) \quad \text{(Formula 1)}$$

ΔV

where:

K = Friedenwald's Coefficient of Scleral Rigidity

P_1 = Intraocular pressure resulting from intraocular volume increment, V_1

P_2 = Intraocular pressure resulting from intraocular volume increment, V_2

$$\Delta V = (V_2 - V_1)$$

thus, ΔV represents an absolute increment in volume without regard to the magnitude of the initial intraocular volume.

This coefficient of scleral rigidity, K was used by Friedenwald as a measure of the structural rigidity of the eyeball. Accordingly, eyes having a high value for this coefficient were considered to have a greater resistance to deformation due to an increased structural rigidity in their walls. Conversely, eyes having a low value for this coefficient were more structurally pliable and hence would show less resistance to deformation. This relationship was used to extrapolate open-stopcock data to zero volume in the calibration of the Schiötz tonometers³ and is an extremely important factor in the estimation of the intraocular pressure by indentation tonometry.

In deriving the formula for his rigidity coefficient, Friedenwald assumed the initial volume of the eye to be "roughly constant" and dealt only with increments of volume and their effect on the intraocular pressure.³ The work of other investigators^{1,2} suggests that this assumption is unwarranted and indicates that the present formula of Friedenwald for the coefficient of scleral

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rigidity, as an absolute and comparative measure of the structural pliancy or rigidity of the ocular coats, is unsound because it omits V_0 , the initial intraocular volume from the relationship.

The purpose of this paper, therefore, is to present experimental data on pressure-volume relationships in cat eyes of varying initial intraocular volumes and to offer a modification of Friedenwald's formula for measurement of a coefficient of scleral rigidity which takes the initial intraocular volume into consideration and demonstrates that the actual structural rigidity of the ocular coats seems to play a minor role in variations of Friedenwald's coefficient between normal eyes in a given species.

MATERIAL AND METHODS

Eleven kitten and cat eyes were used for this experiment. The eyes varied in initial intraocular volume from approximately 0.9 cc. in the smallest to approximately 6.4 cc. in the largest. For approximation of the intraocular volumes the three major diameters of all eyes were measured with a micrometer. The mean of these three diameters was taken as the average value for the diameter of the eye. One millimeter was deducted from all average diameters as an overall allowance for thickness of the ocular coats. One-half of this value was then taken for the radius of each eye, considered for purposes of simplicity as a thin-walled fibro-elastic spherical envelope enclosing an

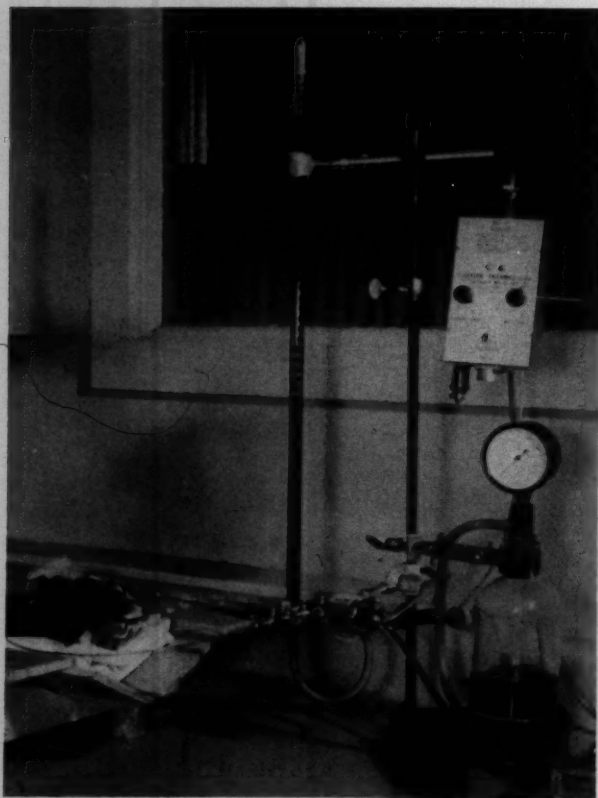


Fig. 1 (Sampson and Girard). Experimental apparatus used to determine the effect on the intraocular pressure from a measured volume increment.

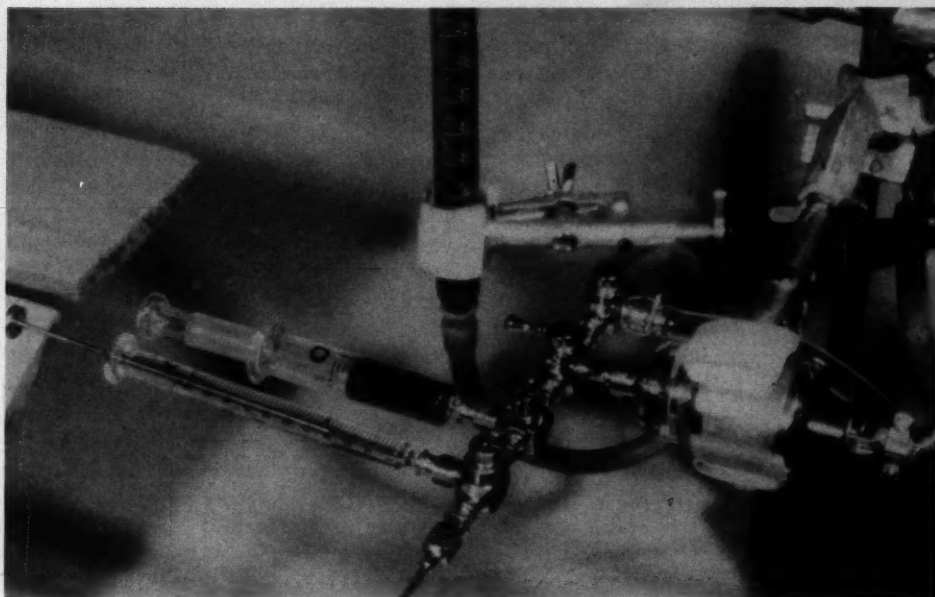


Fig. 2 (Sampson and Girard). Close up of apparatus showing microliter syringe used for volume increments; stopcock arrangement with strain gauge; and water manometer used for calibration.

incompressible fluid. The volumes were then calculated from the formula for volume of a sphere, i.e.,

$$V = \frac{4}{3} \pi r^3 \quad (\text{Formula 2})$$

The animals were sacrificed with an overdose of sodium nembutal and the eyes immediately removed and measured for volume as described above. They were then supported in a small dish packed with cotton moistened with saline and cannulated through the anterior chamber with a 23-gauge needle. This needle was connected to an E. & M. P500 strain gauge (the equivalent of a Statham P23A strain gauge) which acted as a pressure transducer. The connection between the needle and transducer was through a rigid piece of polyethylene tubing and a series of three-way stopcocks. The entire system then was fluid-filled and checked for leaks. The strain gauge was connected to a carrier preamplifier and demodulator

which utilized a carrier frequency of 5000 cycles per second. This was fed into a direct coupled push-pull pen driver amplifier which was used to drive a d'Arsonval type moving coil recording pen. This apparatus is pictured in Figures 1, 2, 3 and is one of the many Physiograph circuits available in the Department of Physiology and Laboratory of Biophysics at the Baylor University College of Medicine.⁴ It is similar in purpose to the one initially described by Grant and Trotter⁵ for the type of measurements conducted in this procedure.

The volume increments were introduced into the system by a Hamilton microliter syringe connected to one of the three-way stopcocks, Fig. 2. Multiples of 2 mm³ of solution were rapidly injected into the system which was considered to be absolutely rigid except for the distensibility of the eye and the very minute movements of the strain gauge diaphragm.

The procedure followed was to inject the

measured volume increments into the eye strain-gauge system by means of the microliter syringe. The pressure increments were then recorded on a curvilinear scale by the pen recorder. The strain-gauge was calibrated against a water manometer and the measurements transposed into millimeters of mercury. The eye, strain-gauge system and pressure calibrating manometer were all set at the same level before any measurements were taken. The intraocular pressure of all eyes was adjusted to at least 10 mm Hg at zero volume and was never stressed above 40 mm Hg at final volume in order to avoid errors due to hypotony and over-distention with its consequent elastic "set" and leakage.

There were no corrections made for: loss of fluid to or from the system; extrapolation of injection to zero time; or for curvilinear displacement of the recording pen. Although this may impair the absolute accuracy of the results, it will not invalidate the conclusions to be drawn from this work.

This study is attempting to show in a semi-quantitative manner the relationship between a variation in the initial intraocular volume; its effect on pressure-volume increments; and discrepancies in current concepts of evaluating these variables. Finally a modification in present methods of quantifying these variables is offered which will hold for eyes of all initial volumes. This is a groundwork upon which more accurate studies will be performed in the future.

The data obtained from the measurements described were analyzed first according to the standard method of Friedenwald in which the *absolute* value of the volume increments were plotted against the logarithm of the pressure increments. The points were plotted on semi-logarithmic coordinates in which the pressure was a common logarithmic scale on the ordinate and the volume increment a linear scale on the abscissa. The best straight line through all of the points was then taken as the average value for the

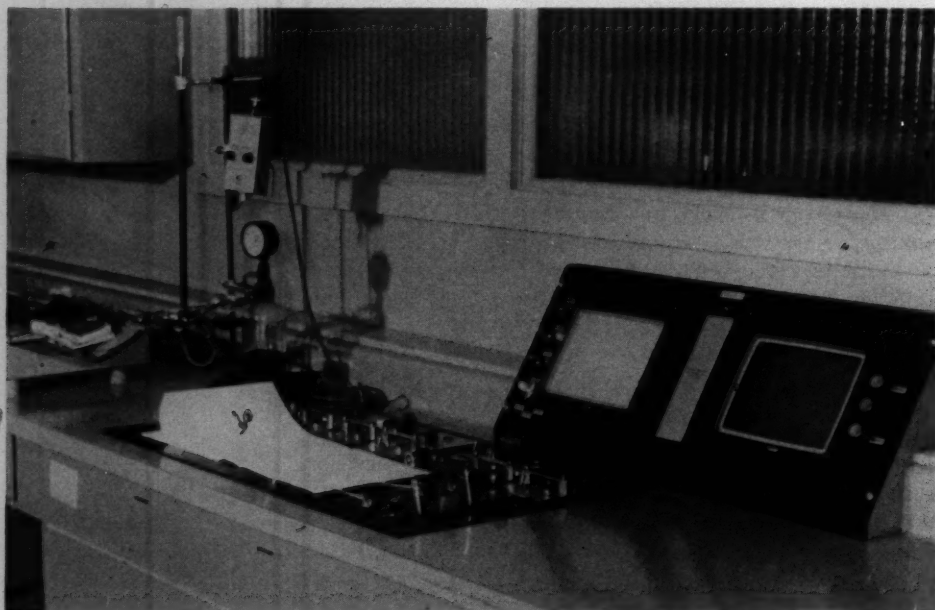


Fig. 3 (Sampson and Girard). Physiograph recorder used in association with the volume increment apparatus to record the effect on the intraocular pressure from a measured increment in volume.

scleral rigidity for the range of pressures in which the measurements were made. The slope of this line was calculated from Formula 1. This, of course, ignores the initial volume and leads to unexpected results. However, if the volume increment in Friedenwald's formula is treated as a *percentage* of the total volume of the eye under consideration, the initial volume is now considered in the relationship. A second analysis of the data with this percentage volume increment, $V\%$, showed the existence of a much closer relationship between eyes of differing initial volumes. Therefore, Friedenwald's formula was modified as follows:

$$K_v = \log_{10} \left(\frac{P_{t_2}}{P_{t_1}} \right) \quad (\text{Formula 3})$$

$$\Delta V\% \times 100$$

where

K_v = Coefficient of scleral rigidity, corrected for variation of intraocular volume, (Volume corrected scleral rigidity).

P_{t_1} = Intraocular pressure resulting from percentage volume increment, $V_1\%$

P_{t_2} = Intraocular pressure resulting from percentage volume increment, $V_2\%$

$$\Delta V\% = (V_2\% - V_1\%)$$

$\Delta V\%$ then actually represents the increment in volume of the eye under stress which produced the pressure change from P_{t_1} to P_{t_2} much the same as it does in Formula 1; however, it is now expressed as a percentage increment of the total volume. For example: a volume increment of 4 mm³ into an eye with an initial volume of 1170 mm³ represents a $V\%$ of .34%; a volume increment of 20 mm³ into an eye of 4860 mm³ initial volume represents a $V\%$ of .41%. The advantage of comparisons of percentage volume change versus absolute volume increment will be discussed.

RESULTS

The results of the pressure-volume studies on the eleven eyes are summarized in Table

TABLE 1

COMPARISON OF VALUES FOR THE COEFFICIENT OF SCLERAL RIGIDITY FOR EYES (11 CATS) OF VARYING RADIUS OF CURVATURE, BEFORE AND AFTER A CORRECTION FOR VARIATION OF INTRAOCULAR VOLUME

Scleral Rigidity, K	Radius of Curvature in mm.	Volume in cc.
0.0595	6.0	0.90
0.0523	6.5	1.17
0.0521	6.5	1.17
0.0476	6.25	1.03
0.0158	9.0	3.06
0.0155	8.5	2.60
0.0113	11.0	5.60
0.0105	10.5	4.86
0.0100	10.5	4.86
0.0098	11.5	6.40
0.0078	10.0	4.20

Volume Corrected Scleral Rigidity, K_v	Radius of Curvature in mm.	Volume in cc.
0.0065	6.5	1.17
0.0063	6.5	1.17
0.0057	6.25	1.03
0.0056	11.5	6.40
0.0055	11.0	5.60
0.0054	6.0	0.90
0.0050	9.0	3.06
0.0050	10.5	4.86
0.0049	10.5	4.86
0.0045	8.5	2.60
0.0033	10.0	4.20

(Sampson)

1 which shows a comparison of numerical values for the average coefficient of scleral rigidity for eyes of varying radii of curvature (and hence varying initial intraocular volumes). The wide spread between the highest and lowest values for the scleral rigidity, K , is compared with the close agreement between the highest and lowest values for the volume corrected scleral rigidity, K_v . When compared on semi-logarithmic coordinates the values obtained for the scleral rigidity, K , calculated from Formula 1, show a large variation in their slopes with the smaller eyes showing the higher values or greater slopes (fig. 4). Comparison of the volume corrected scleral rigidities, K_v , calculated from Formula 3, shows a better correlation, however, with some of the smaller eyes again showing the greater slopes (fig. 5). With the exception of the

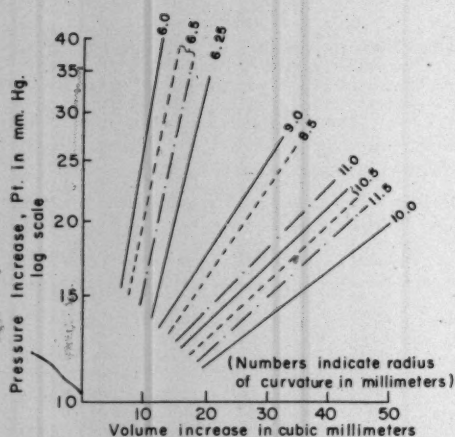


Fig. 4 (Sampson and Girard). Coefficient of scleral rigidity K of eleven cat eyes of varying sizes.

one eye (radius of curvature 10.0 mm.) showing a slope apart from and much lower than the rest, the agreement in the latter comparison is very good. (This same eye had the lowest value for K and K_v and represents either an eye with abnormally great pliancy of its walls or a gross leak in the system.)

Two of the eyes were selected for a closer analysis to further the point of a variation in the intraocular volumes. One was a kitten eye with a radius of curvature of 6.5 mm and an initial volume of approximately 1170 mm³; the second an adult cat eye with a radius of curvature of 10.5 mm and an initial volume of approximately 4860 mm³. Figure 6 shows a comparison of the pressure-volume relationships between these two eyes, with sample tracings copied from actual data recorded on the Physiograph; and a comparison of a calculated value for the coefficient of scleral rigidities before and after a correction for the variation in intraocular volume. In the eye with a radius of 6.5 mm it takes only 10 mm³ of volume increment to increase the intraocular pressure from 11 mm Hg to 33 mm Hg, whereas in the eye with a radius of curvature of 10.5

mm it takes 40 mm³ of volume increment to get the same pressure increment. The second eye has a little more than four times the initial intraocular volume than does the first eye, and, as seen in the data, it takes four times the volume increment in the second eye to cause the same pressure increment over the same range of intraocular pressure in both eyes. A comparison of the scleral rigidities calculated from Formula 1 shows a value for K of 0.0521 in the smaller eye and a value for K of 0.0100 in the larger eye, a very large variation. However, when compared from calculations from Formula 3, K_v for the smaller eye is .0065 with a value of .0049 for the larger eye. The much closer correlation between the two in this latter comparison is obvious.

Figure 7 shows a comparison of scleral rigidities, K , of these two eyes on semi-logarithmic coordinates and demonstrates graphically the variation. Figure 8 shows a comparison of the volume corrected scleral rigidities, K_v , of the two eyes on semi-logarithmic coordinates and graphically reveals the good correlation.

Finally, Figure 9 shows a comparison of

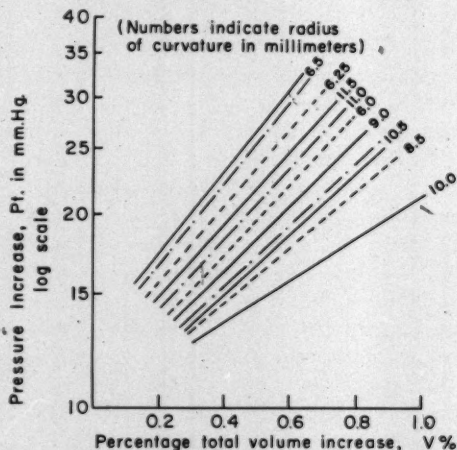
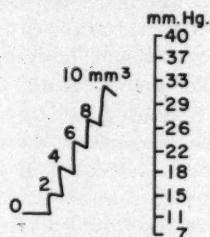


Fig. 5 (Sampson and Girard). Coefficient of scleral rigidity K_v of eleven cat eyes of varying sizes corrected for variations of intraocular volume.

$$K = .0521$$

$$K_v = .0065$$

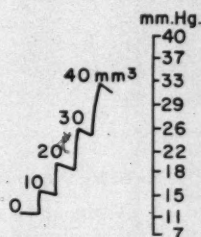
radius = 6.5 mm.
volume = 1170 mm³



$$K = .0100$$

$$K_v = .0049$$

radius = 10.5 mm.
volume = 4860 mm³



Percentage Volume Increase, V%

2 mm³ = .17 %
4 mm³ = .34 %
6 mm³ = .51 %
8 mm³ = .68 %
10 mm³ = .85 %

10 mm³ = .20 %
20 mm³ = .41 %
30 mm³ = .61 %
40 mm³ = .82 %

(Sampson)

Fig. 6 (Sampson and Girard). Comparison of the pressure-volume relationships between two eyes of different volumes; with a comparison of a calculated value for the coefficient of scleral rigidities before and after a correction for the variation in intraocular volume.

K and K_v for these two eyes on the same semi-logarithmic coordinates. Although the two functions for the eye with radius of 10.5 mm have almost identical slopes, they differ in their numerical value. This is explained on the basis of different linear values of the abscissa for each function when plotted against the same ordinate. K will always have a larger numerical value than K_v , because its abscissa has smaller numerical values per unit length when compared to the abscissa for K_v . This figure is shown to point out that values of K and K_v cannot be compared directly but only through a constant, (which is a factor of two when the abscissas are related to each other as they are in Figure 9).

DISCUSSION

The known inverse relationship between the initial intraocular volume and the change in intraocular pressure produced by a given

increment in that volume has been substantiated in this experimental work on eleven cat eyes of varying initial intraocular volumes. When Friedenwald's classical formula for the coefficient of scleral rigidity, Formula 1, was used to analyze the experimental data it was found that as the eyes diminished in size the calculated value for the rigidity coefficient, K , rapidly increased. This is explained by the fact that as the initial intraocular volume diminished the same volume increment produced a correspondingly greater stress on the ocular coats and hence a greater change in pressure per unit volume.³ Since these smaller eyes were also the eyes of younger animals of the same species in which the actual structural rigidity of the scleral sacs is qualitatively known to be much less than that of the older animals as evidenced by a comparison of intact and eyiscerated cat and kitten eyes shown in Figures 10 and 11, it becomes obvious that

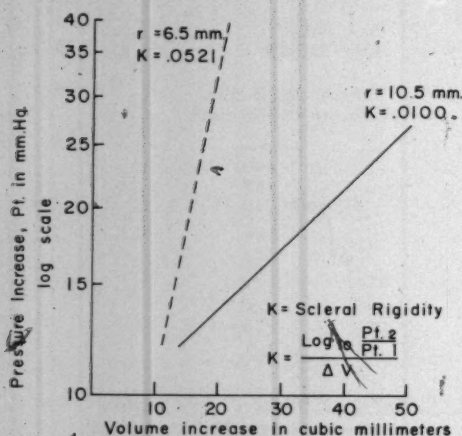


Fig. 7 (Sampson and Girard). Comparison of the scleral rigidities of a kitten eye with a radius of 6.5 mm and a cat eye with a radius of 10.5 mm.

the coefficient of scleral rigidity is not a direct measure of the structural pliancy of the scleral walls. It is merely a *mathematical constant* between physical variables of pressure and volume and, if used to define the elastic properties of the globe per se, the result will be misleading.

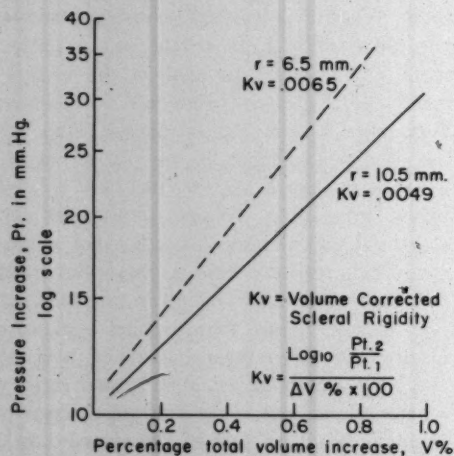


Fig. 8 (Sampson and Girard). Comparison of the volume corrected scleral rigidities of a kitten eye with a radius of 6.5 mm and a cat eye with a radius of 10.5 mm.

When the data was analyzed using the modification of Friedenwald's formula designed to correct for variations in the initial intraocular volumes, Formula 3, a much closer agreement for the calculated values of the volume corrected rigidity coefficients, K_v , was found, however, with some of the smaller and structurally more pliable eyes again showing the larger values. Here as the intraocular volume decreases the volume increment is reduced in proportion so that a more uniform stress is exerted upon the ocular coats with a resulting more uniform change in pressure per unit of percentage volume increment. The probable explanation of the smaller eyes again showing the larger values is that, with the technique employed, there is a much greater chance of experimental error as the eyes decrease in initial volume. If a more careful analysis with more sensitive apparatus is performed, it is suggested that within the limits of experimental error, all normal eyes of a given species of varying intraocular volumes will show the same constant of proportionality

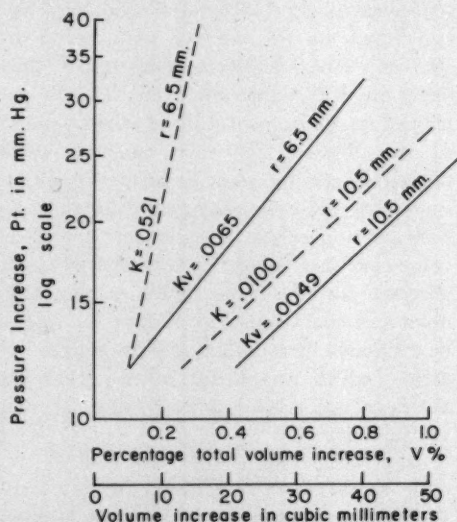


Fig. 9 (Sampson and Girard). Comparison of the scleral rigidities before and after a correction for the variation in the intraocular volume.

between pressure and volume increments, if a correction is made for variations in the intraocular volume as suggested, and assuming that the measurements are made over the same range of average normal intraocular pressure for all eyes (the normal range of intraocular pressure being defined herein as a Pt of not less than 10 mm Hg, nor more than 40 mm Hg). It should be pointed out that this volume corrected scleral rigidity is merely a variant of Friedenwald's method and is still just a constant of proportionality between transient pressure and volume increments. While it apparently serves the purpose of providing a closer correlation between these increments and variations in the initial intraocular volume it cannot be used to directly quantitate the elastic properties of the sclera within a given species.

On the basis of this study and the work of others it is suggested that the effect of the structural pliancy or rigidity of the scleral walls on the coefficient of scleral rigidity, when measured over normal pressure ranges, is of less significance than was previously thought. On the contrary, variations in the initial intraocular volume seem to constitute the most important single physical factor in the variation of the coefficient of scleral rigidity between normal eyes. The variation in the intraocular volume, therefore, is not a variable apart from the coefficient of scleral rigidity, but is an integral factor in this function when calculated according to

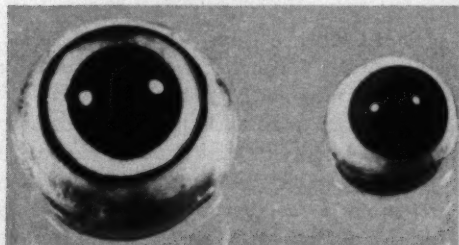


Fig. 10 (Sampson and Girard). Comparison of an intact cat eye of 10.5 mm radius with that of an intact kitten eye with a radius of 6.5 mm.

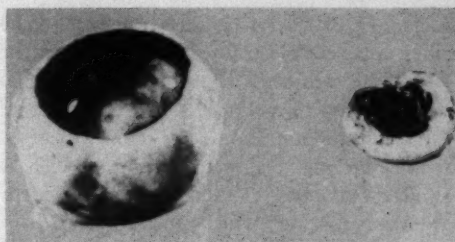


Fig. 11 (Sampson and Girard). Comparison of these same eyes after evisceration. Notice the collapse of the young kitten eye indicating a lack of structural rigidity as compared to that of the older cat eye which maintains its overall spherical shape after removal of its viscera.

Friedenwald's formula. Friedenwald's assumption of a "roughly constant" initial volume of the eye in arriving at his relationship for quantitating the structural rigidity of the ocular coats is therefore definitely unwarranted, and many of the corrections for scleral rigidity used in clinical ophthalmology during tonometry and tonography are not correcting for differences in the structural pliancy of the scleral walls but are adjusting for variations in the initial intraocular volume. To be sure, there are other factors that are known to influence the scleral rigidity coefficient and its variation from what is considered normal. Becker,⁶ Weekers,⁷ and Drance⁸ have all pointed out various metabolic and endocrine factors which will change the scleral rigidity in a given eye. It is well known that in some eyes following surgery and trauma the coefficient of scleral rigidity will become altered. It is conceivable that the influence of these other factors on the coefficient of scleral rigidity might possibly be explained, in part at least, by fluctuations in the initial intraocular volume due to expansion or contraction of the scleral envelope, since by and large a departure of the coefficient of scleral rigidity from the mean value, when calculated with Friedenwald's equation, is a reflection of variations in the volume of the eye. For example, a hyperopic eye is likely to show an increased

coefficient of scleral rigidity if it is an axial ametropia, not because of an increase in the structural rigidity of its walls but due to the fact that its volume is less than that of the standard emmetropic eye upon which the tonometer was originally calibrated.^{1,2,3} A similar but reverse situation will hold for axial myopia in which the coefficient of scleral rigidity is often found to be lower than the average value. However, it should be pointed out that since a given ocular refraction can be associated with a wide range of axial lengths,⁹ an unusually small intraocular volume does not necessarily imply hyperopia, or vice versa. Thus the absence of a significant refractive error does not eliminate the possibility of variations in the intraocular volume influencing the coefficient of scleral rigidity and its role in estimating the intraocular pressure.

CONCLUSIONS

It is not to be inferred that the distensibility or pliancy of the ocular coats plays no role in the measurement of the constant between volume and pressure increments but only that, as pointed out, it is much less important than generally conceived as a cause for the variation of this constant between normal eyes. In this study at least, cat and kitten eyes of markedly different structural properties showed surprisingly close correlation between transient volume and pressure increments when stressed in normal pressure ranges and the increments adjusted for variations in the initial intraocular volume. It may be speculated that the role of the distensibility of the ocular coats per se, is more important in comparisons between eyes of different species than between eyes of a given species but of different sizes.

Other applications in clinical ophthalmology of a variation in the intraocular volume from the normal value have been adequately discussed by Phillips and Quick in their excellent article.¹ Although it is not within the scope of this paper to discuss this topic completely, it might be mentioned that

the use of Goldmann's applanation tonometer for measurement of the intraocular pressure will almost eliminate the effect of the coefficient of scleral rigidity, as with the small applanation of the cornea the displaced volume or indentation is considered to be insignificant and for all practical purposes P_t equals P_o .¹⁰ Thus errors in the estimation of intraocular pressure by indentation tonometry due to variations in the initial intraocular volume are best revealed and corrected by the use of the applanation tonometer. However, since for many reasons the applanation tonometer will probably never attain the widespread clinical use enjoyed by the indentation tonometers, it is important to understand the many variables that can effect a tonometric reading, foremost of which is the influence exerted by the so-called coefficient of scleral rigidity. The role of this constant and the factors which modify it should be clearly understood.

SUMMARY

In arriving at his formula for the coefficient of scleral rigidity, Friedenwald assumed the initial volume of the eye to be a constant and considered only increments of volume and their effect on the intraocular pressures in his relationship. This study, and the work of other investigators, suggests that this assumption is unwarranted and indicates that the present formula of Friedenwald for the coefficient of scleral rigidity, as a measure of the structural pliancy of the ocular coats, is unsound because, among other reasons, it omits as a variable the initial intraocular volume.

This paper presents experimental data on pressure-volume relationships in eleven cat eyes of varying initial intraocular volumes and offers a modification of Friedenwald's formula for the coefficient of scleral rigidity which considers the initial intraocular volume as a variable. Analysis of the data with this formula inferred that, within the range of intraocular pressures of clinical

importance, the structural pliancy of the scleral walls seemed to play an insignificant role in a calculated value for a coefficient of scleral rigidity, the major factor being a variation in the initial intraocular volume.

This study indicates that the effect of a variation of the initial intraocular volume is not a factor apart from Friedenwald's coefficient of scleral rigidity but is an integral part of its value. In the absence of a few metabolic and endocrine factors which are known to somehow influence the coefficient

of scleral rigidity, a departure of apparently normal eyes from the mean value, when calculated with Friedenwald's equation, is a reflection of variations in the volumes of these eyes. As a consequence, these variations in initial volumes must replace many of those attributed to the structural pliancy of the scleral walls which to date have been held almost entirely responsible for discrepancies between the findings of applanation and indentation tonometry.

Division of Ophthalmology.

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THE EFFECT OF RESTRICTED VISUAL SPACE ON THE PRIMATE EYE*

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INTRODUCTION

Since 1864 and 1865 when Donders¹ concluded that the principal cause of myopia was tension of the eyes for nearwork and Cohn² proposed that "school myopia" is caused by excessive nearwork in the growing child, a great deal of controversy has

arisen concerning the validity of this proposal. No satisfactory evidence for or against this proposal has been accumulated since the increase in incidence and amount of myopia during the school years found on many surveys may be explained just as well by an hereditary-growth hypothesis as by the nearwork hypothesis.

Because of the problems involved in the use of human subjects for an experimental attack on the problem of myopia development, no studies have been made on humans. However, a number of studies were made

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between 1910 and 1935 by Levinsohn and his supporters and by his detractors. Levinsohn^{3, 4, 5, 6, 7, 8, 9} made a series of studies using rabbits, cats and dogs to prove that the pull of gravity on the eye and the increased blood flow to the eyes when the face is held in a horizontal position are responsible for the development of myopia. The early experiments on cats, dogs, and rabbits were not particularly successful, and he later turned to the use of primates.

When young monkeys were enclosed in boxes with their heads exposed, and the boxes were positioned to keep the animals' faces horizontal with the eyes downward for a period of six hours a day six days a week for at least three months, the monkeys developed myopia and demonstrated changes in refraction of up to seven diopters. The onset of refractive changes was not immediate but required a month or more before it began. The refractive changes were not the same for all animals and not all animals demonstrated refractive changes (various groups had between 60 and 100 percent of the animals with refractive changes). After onset the changes continued as long as the animals were kept in the experimental situation. No information was presented as to any further changes after the monkeys were removed from the experimental situation. No refractive changes occurred in control animals maintained in the boxes but in an upright position for the same period of time.

Similar results were obtained by Essed and Soewarno¹⁰ and by Marchesani.^{11, 12} The latter investigator found refractive changes occurring in his control animals which were allowed to remain in the living cage rather than being placed in the boxes. Even though the changes in refraction were greater in the experimental animals than in the control animals, Marchesani¹² felt that the number of animals involved was not sufficient to support Levinsohn's position in view of the changes which did occur in the control animals.

In order to keep the face of the monkey

horizontal it was necessary to elevate the body above the head which results in increased intraocular pressure and weakens Levinsohn's test of the gravity theory which is questionable on still other grounds. The development of refractive changes by this procedure was ignored by Levinsohn's critics who concentrated on deriding the gravity theory on both theoretical¹³ and practical grounds^{14, 15} (no monkey in his normal environment would spend six hours a day in this position). However, the consistent and controllable induction of refractive change by any manipulation of the environment is of great importance to the study of the development of myopia and refutes the extreme position taken by some proponents of the heredity theory that environment has no effect on the development of myopia. This is the important conclusion to be drawn from Levinsohn's studies as he himself points out¹⁶ and it must not be hidden by the controversy over the gravity theory.

METHODS

Levinsohn obtained his most consistent results by using monkeys which were between six and eighteen months old when the study started. In the present study the *Macaca nemestrina* (pigtail) monkeys were between four and six years of age; in fact two subjects were removed from the study because they gave birth to single offspring while serving as subjects. Thirteen animals, five males and eight females, were available at the start of the study. Nine of these animals were selected at random as the experimental group and the remaining four animals were to serve as a control group. Accidents and disease reduced the control group to one animal before the study was well underway. A second group of nine adolescent monkeys, three and four years old, was obtained and served as the control group.

The nine experimental animals were placed in restraining chairs of the type developed by Mason¹⁷ and modified by Young¹⁸ as shown in Figure 1A. The chairs

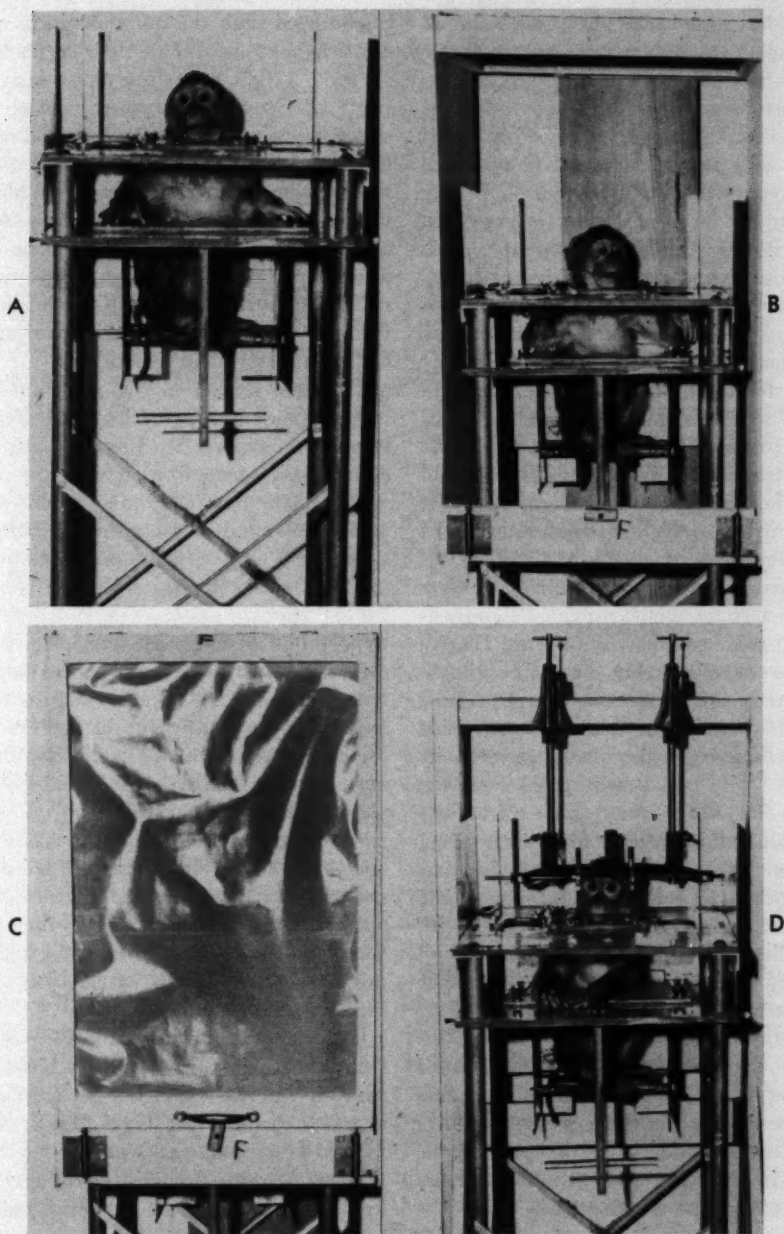


Fig. 1 (Young). A-Restraining chair, B-Restraining chair in hood, C-Hood in closed position, D-Monkey in position for refraction.

are 16 inches wide by 20 inches deep and 32 inches high. The under side of the lower plate was painted white and served to prevent vision downward. The upper part of each chair was enclosed in a hood made of plywood with an open top and front which were covered with architect's cloth to provide light but prevent vision as shown in Figure 1B and 1C. The inside of the hoods were painted white except for the rear panel which was formed by the flat black wall of the room. The hoods were not more than twenty inches from the eyes of the monkey at the furthest point and averaged around 14 inches. The chairs and hoods were arranged under a 160 watt fluorescent unit to provide four footcandles of illumination on a point midway between the two plates of the chair and ten inches in front of the centerline of the monkey.

For refraction the chair and monkey were placed in a frame which provided an adjustable head-holder as shown in Figure 1D. All animals were refracted in the chair throughout the study. All refractions except the final refraction were made under homatropine and paredrine cycloplegic as suggested by Harrison¹⁹ with one drop instilled every ten minutes for four doses. The refractions were made within an hour to an hour and a half after the administration of the first dose. Further, all refractions throughout the study were made by the same individual with two readings of each eye being taken with the aid of a modified Wolfe Ski-Optometer which permitted one hand operation or by the use of trial lenses.

While the head-holder prevented head movement, it did not prevent eye movements which are sufficient in the monkey to require a considerable patience on the part of the operator in order to get reliable results. During the early part of the study while the monkeys were still naive, it was possible for an assistant to hold their attention while the finding was taken. However, since these maneuvers were not successful during the latter part of the study, the monkeys were

tranquilized with the administration of one and a half milligrams of Sparine per pound of body weight given intramuscularly. The effect of Sparine on refraction was determined by maintaining cycloplegia and refracting the animals before and after the administration of Sparine allowing an hour for the Sparine to take effect. It had no measurable effect. All findings were taken to the nearest quarter of a diopter. Only two animals demonstrated astigmatism and this less than $\frac{3}{4}$ diopters. The astigmatism was recorded as equivalent sphere.

The final refraction was made under the homatropine and paredrine cycloplegic as were all the other refractions and also under a three day atropinization with the administration of one drop of a one percent aqueous atropine sulfate solution three times a day for a total of ten doses. The effects of the different cycloplegics may be compared for 14 animals or 28 eyes. No differences were found between the cycloplegics in 12 eyes, the refraction was $\frac{1}{4}$ diopter more plus under atropine in 15 eyes and $\frac{1}{2}$ diopter more plus under atropine in one eye. Thus there is only one chance in 28 that the finding under homatropine and paredrine cycloplegic would show more than $\frac{1}{4}$ diopter more minus than the finding taken under atropine. All results presented were taken under the homatropine and paredrine cycloplegic but are similar enough to the findings taken under atropine to rule out spasm of accommodation as an explanation of the changes which have occurred.

The experimental animals were removed from the chairs and hoods at biweekly or monthly intervals for two day periods and returned to the living cages. They were refracted every two weeks except for the first refraction which occurred at the end of the first month. Of the nine experimental monkeys two females were removed at the end of four months because of births and one male was removed at the same time because of illness. The remaining six animals continued in the study for one year and a total

TABLE 1

MEAN AND MEDIAN REFRACTIONS AND STANDARD DEVIATIONS IN DIOPTERS FOR SIX MONKEYS IN THE RESTRICTED VISUAL SPACE SITUATION ELEVEN MONTHS

Measure	Months under hoods											
	0	1	2	3	4	5	6	7	8	9	10	11
Mean	-0.33	-0.48	-0.71	-0.83	-0.90	-1.00	-1.10	-1.06	-1.08	-1.10	-1.10	-1.10
Median	-0.33	-0.34	-0.69	-0.71	-0.88	-0.88	-1.00	-0.87	-0.87	-0.87	-0.87	-0.87
S.D.	0.28	0.31	0.32	0.39	0.49	0.46	0.53	0.54	0.55	0.57	0.57	0.57

time under the hoods of eleven months. The nine control animals were refracted at the beginning and the end of an eight months period which was spent in living cages in a room 8 feet wide by 16 feet long by 9 feet high.

RESULTS

The results obtained on the control group of nine animals show an initial means refraction of .00 diopters and a median refraction of .00 diopters. The refractions at the end of eight months show an average of .19 diopters minus and a median of .125 diopters minus. Through the use of a repeated-measures t test on the same subjects the difference between the pre- and post-refractive mean findings was found to be significant at the 5 percent level of confidence.

The refractive findings obtained on the six experimental animals that completed the study are presented in Table 1. The results are presented for monthly intervals rather than fortnightly intervals in order to save space since the omitted values are in line with the values presented in Table 1 as may be seen from Figures 2 and 3. A comparison of the mean pretest findings on the control (.00 diopters) and experimental (-.33 diopters) groups yields a difference which is significant at the 5 percent level on a random-groups t test.

Figure 2 presents the average refractive findings taken at biweekly intervals for the six animals over the total duration of the study and for all nine animals for a four months period together with the standard deviations. Figure 3 presents the median findings for the same groups.

The t tests between the average pre-experimental refraction and the average refraction at the end of four months, six months and 11 months are significant at the .1 percent level of confidence which indicates that these results are extremely unlikely to be due to chance factors operating in the experimental set-up. The differences between the results obtained on the experimental and control groups at six months and at eight months are equally significant.

DISCUSSION

Since the results are much the same in both figures and all comparisons of a statistical nature relate to the mean refractive results, the discussion will be based on Figure 2. The refractive changes which occur in the restricted visual space situation fit a smooth function which is the same whether it is based on biweekly or monthly measures. The curves shown in Figures 2 and 3 tend to start slowly for the first month and then drop more rapidly for the following five months when the maximum effect is reached. The curves become asymptotic beyond the sixth month. The slow start and the rate of change are generally similar to the results found by Levinsohn. However, his animals continued to show refractive changes as long as they remained in the experimental situation rather than leveling off at the end of six months with no further change occurring in five additional months in the situation.

The curves based on all nine experimental animals over the four months period parallel those based on the six subjects who completed the study. Thus the changes occur-

ring in the three subjects dropped from the experimental group are similar to those occurring in the remaining six monkeys, and the results may be generalized to all nine subjects. The standard deviations presented in Table 1 and Figure 2 indicate that all animals changed at about the same rate for the first two months since the standard deviations are approximately the same. During the next four months the standard deviations increase in size and then level off again after the sixth month. This increase in the size of the standard deviation indicates different rates of change among the animals with stabilization after six months.

While all animals showed some change, two animals had a small change ($\frac{1}{4}$ - $\frac{1}{2}$ diopter minus) which was maintained over the whole period while the other seven showed changes in excess of one half diopter minus

which increased but leveled off after six months in the experimental situation. If the small changes are ignored, roughly $\frac{3}{4}$ of the animals in the experimental situation showed minus changes. This proportion is similar to that obtained in the studies discussed earlier. Using the same standards for the control animals, one animal out of nine showed refractive changes in excess of $\frac{1}{2}$ diopters minus. The changes found in the control group are similar to those found by Marchesani who kept his control animals in living cages.

The refractive changes found in this study may be compared with those found on humans and the results obtained by Levinsohn. The average change of $\frac{3}{4}$ diopter in six months found in the present study exceeds the usual rate of change found among human progressive myopes but falls short of

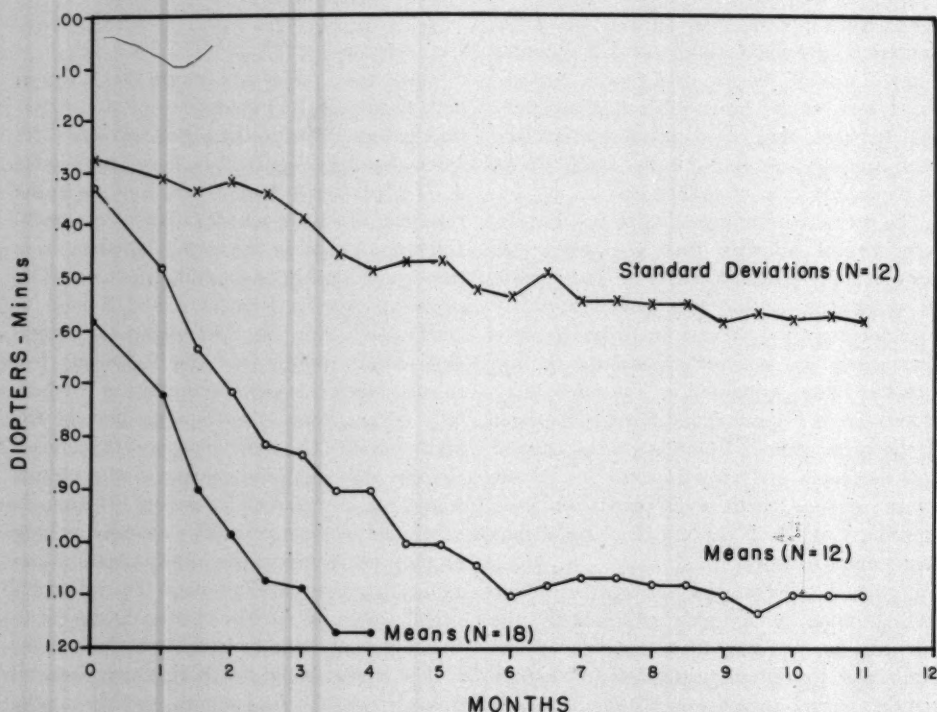


Fig. 2 (Young). Mean refractive changes and standard deviations in 12 eyes during 11 months and in 18 eyes, including the 12, during four months in a restricted visual space.

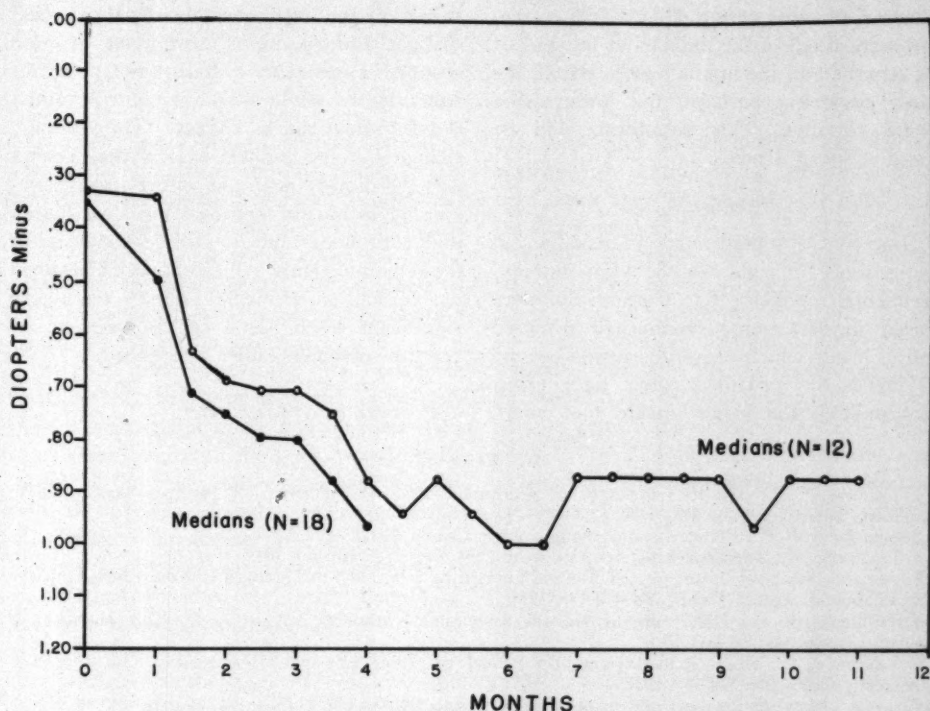


Fig. 3 (Young) Median refractive changes in 12 eyes during 11 months and in 18 eyes, including the 12, during four months in a restricted visual space situation.

the changes recorded by Levinsohn. From Levinsohn's discussion of the Essed and Soewarno results it appears that of seven animals kept in boxes for one year the average change occurring in five animals which showed changes is approximately 3 diopters. Clearly this change is much greater than the change occurring in the present study.

Two important differences may be noted between these studies: the age of the animals and the positions used. Levinsohn used very young animals, $\frac{1}{2}$ to $1\frac{1}{2}$ years old, whereas animals four to six years old were used in this study. Roughly, the monkey's developmental age may be compared with humans three times as old. Using this basis his subjects were comparable to humans one and a half to four and a half years old whereas the monkeys used in this study compare to humans twelve to eighteen years old. On age

alone one would expect more change to occur in his animals than in the animals used here. His position with the body elevated above the head introduces pressure changes which are likely to be much more severe than the changes (if any) which occur in the vertical position used in this study.

Since the animal can only see the parts of his body and the chair when enclosed within the hood, he must converge and accommodate if he looks at anything. This nearwork is not exactly comparable to that performed by humans when reading or doing work which requires concentration. It is possible that a greater change would have occurred if a closer approximation to the human nearwork situation could have been obtained. The stabilization at the end of six months suggests that the eye adapts itself to the situation and remains adapted as long as the

situation remains constant. If a full correction were fitted to the animals at the end of six months and the animals were left in the hood situation, perhaps the progression would continue. This hypothesis will be tested in future studies.

SUMMARY

Nine control pigtail monkeys remained in living cages for eight months while nine experimental monkeys, 4 to 6 years old, were placed in restraining chairs and enclosed within hoods which prevented vision beyond 20 inches but provided ample light (four footcandles) for vision inside the hoods.

While all the experimental animals showed changes toward minus, seven out of nine changed more than a half diopter within four months while only one control animal showed this much change. The average change for six animals over a year period was $\frac{3}{4}$ diopter with the entire change occurring within six months after being placed under the hoods and no change occurring in the remaining time. All comparisons between control and experimental animals are highly significant when based on the average refraction obtained under cycloplegic.

Primate Research Center.

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CHOROIDAL VASCULARIZATION IN THE RABBIT

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Studies on the human choroid abound in the earlier literature and the reader is referred to the excellent work of Wudka and Leopold¹ for a comprehensive bibliography. In comparison, despite the early use of the rabbit as an experimental animal, there is little information to be found on the choroid of this animal in the older literature.

In his text describing the complete anatomy of the rabbit Krause² dealt only briefly with the choroid, and Tandler³ was more concerned with the large vessel distribution in the head, rather than with fine details. In their experimental studies on ocular blood flow Wagenmann⁴ and Siegrist⁵ commented on some grosser aspects of choroidal vascularization. However, some details of the fine vascular anatomy of the rabbit choroid have been known for many years, due primarily to the work of Virchow⁶ and Leber.⁷ The choroidal vessels received little further attention until the recent work of Vilstrup,⁸ Wudka and Leopold^{1,9} and Scullica.^{10,11}

The present study is part of a wider anatomical investigation into the whole of the vasculature of the rabbit eye and orbit. The principal technique used is similar to that used by Vilstrup and Scullica and comparison with the results obtained by these authors is therefore facilitated.

MATERIALS AND METHODS

A total of 29 adult rabbits were employed in this study, of which four were pigmented and the remainder albino.

The Neoprene latex injection technique used, and the subsequent tissue digestion, has been described in full previously¹² and will not be repeated here. In addition, two of the rabbits were injected with a strong India

ink suspension after flushing the vascular system with normal saline solution and the eyes were then enucleated and fixed in 5 percent formalin.

Before injection, the choroidal vessels were observed in the living animal in some cases. This was done by proptosing the eye in the anesthetized animal and directing a stream of air onto the sclera, having previously cut back the conjunctiva. The sclera became quite transparent under these conditions. Transillumination of these eyes afforded a clear view of the large vessels of the choroid observed through the sclera.

OBSERVATIONS

The Choroidal Arteries: The ciliary artery sends off two long posterior ciliary arteries, one on either side of the optic nerve. They drop below the nerve as they advance towards the eye. Most of the short posterior ciliary arteries branch from the long ciliaries but a few pass directly from the ciliary⁶ artery. The ciliary vessels are joined by the internal ophthalmic artery which usually joins one of the long ciliary arteries or, less frequently, one of their branches. In three preparations, however, the internal ophthalmic artery passed to the retina without anastomosing with the ciliary vessels.

Most of the short posterior ciliary arteries penetrate the sclera approximately in a line which marks the horizontal meridian of the eye. The optic nerve lies well above the posterior pole of the eye, and the vessels therefore enter the eye 3 to 4 mm below it. From two to four smaller vessels pass dorsally to enter the eye adjacent to the optic nerve. In most specimens a few interanastomoses occur between the short posterior ciliary arteries before they reach the eye. In two specimens I observed an aberrant short ciliary artery which passed independently from the

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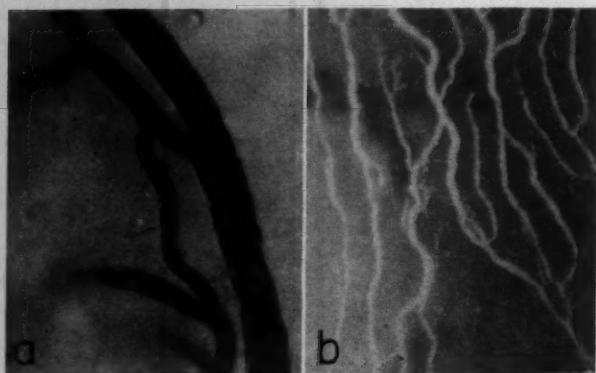


Fig. 1 (Ruskell). a. Anastomosis between branches of the long posterior ciliary arteries within the sclera. $\times 19$ b. Anastomosis between large vessels within the choroid. The dispersion of two short posterior ciliary arteries is shown. $\times 24$

ciliary artery to enter the choroid well forward, in the upper half of the eye. In both cases this vessel used the same route through the sclera as a proximal vortex vein.

The two long posterior ciliary arteries penetrate the sclera obliquely, on opposite sides of the eye in the horizontal meridian. While passing through the sclera, they give off several more branches which also contribute to choroidal vascularization. In most specimens, some of these vessels interanastomose within the sclera (fig. 1a). In the choroid, a few anterior choroidal arteries pass from the long ciliary arteries. The latter divide farther on, to form the major iridic circle. This division may take place in the choroid, ciliary body, or iris and sometimes three branches are formed.

Another source of blood to the choroid is by way of the recurrent choroidal arteries which pass back to the choroid from the major iridic circle. The size of these vessels is variable but generally they disperse over a small area of the choroid. At first these arteries lie external to the veins but they quickly plunge centralwards (fig. 2). They are largely confined to that part of the choroid which lies distal to the vortex centers. A few branches sometimes pass farther back. The majority of these vessels leave the circle near the vertical meridian of the eye i.e. farthest from the two ciliary origins of the circle.

Upon entering the choroid the short posterior ciliary arteries immediately break up into several branches which disperse in every direction, crossing the veins frequently, internally, or externally before passing to an intervenous space where they lie a little central to the veins (fig. 3). Branches from these vessels cross the veins to occupy a neighboring intervenous space (fig. 4). The large veins converge to the vortices and hence, the arteries which lie between them, including the recurrent arteries, do the same.

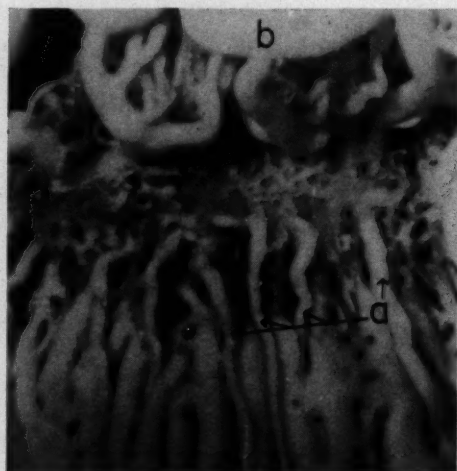


Fig. 2 (Ruskell). Recurrent choroidal arteries (a) from the major iridic circle (b) dispersing in the choroid. In their early course the arteries lie external to the veins. $\times 29$

More anteriorly the intrascleral branches of the long posterior ciliary arteries disperse either in the dorsal or the ventral choroid.

Infrequent anastomoses occur between small branches of the short ciliary arteries and rarely between larger ones (fig. 1b). For the most part, the larger branches of the short ciliary arteries are independent of each other and in the majority of eyes I found no anastomoses at all at this level.

The lumens of the arteries are gradually reduced as they pass anteriorly. The longest of them extend nearly to the anterior border of the choroid at the ora ciliaris retinae. The recurrent arteries are relatively short and rapidly converge to the vortex centers. In no preparation were the recurrent arteries found to anastomose with the posterior ciliary arteries. As the arteries approach the ampullae of the vortices they behave in one of three ways (fig. 5). In most cases the arteries divide and exhaust themselves before reaching the ampullae. Some loop away, frequently reversing their direction. Others



Fig. 3 (Ruskell). Relation between the arteries and veins in the posterior half of the choroid; external view. The arteries, which are narrower than the veins, cross the veins in many places causing constrictions. $\times 37$

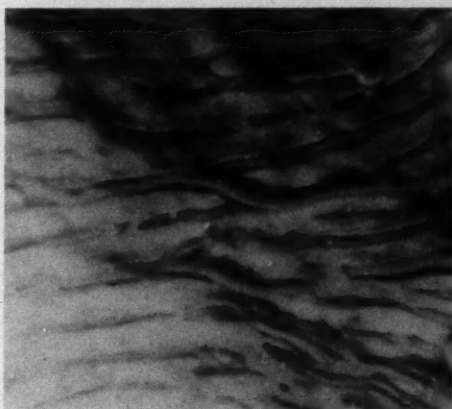


Fig. 4 (Ruskell). Relation between the arteries and veins of the choroid near the equator. $\times 26$

pass forward to cross the ampullae internally, before turning away. In two instances I found direct connections between the looping artery and an adjacent vein. These were both recurrent arteries from the major iridic circle which anastomosed with large veins close to the vortex centers. The largest of them measuring 47 at the point of anastomosis (fig. 6).

The latex casts show an abundance of



Fig. 5 (Ruskell). Arterial pathways at the venous vortex center. The largest artery (a) crosses internal to the ampullae before changing direction. The ampullae has been cut away to reveal the artery (a recurrent choroidal artery). Other arteries (b) turn away before reaching the ampullae. Another artery (c) turns inwards to break up in the choriocapillaris as it approaches the ampullae. $\times 29$

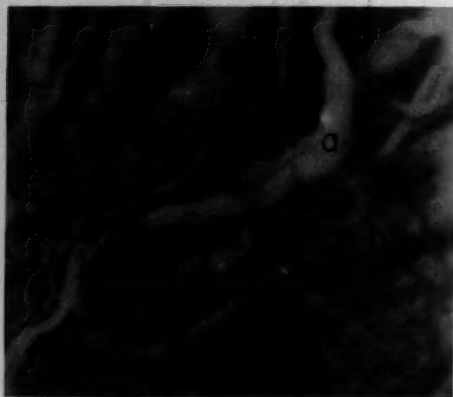


Fig. 6 (Ruskell). A recurrent artery (a) curves away from the vortex center and joins a large vein (b) directly. $\times 29$

venous constrictions where the arteries pass across them (figs. 4 and 5), and this appearance is confirmed by observing the choroidal vessels in the living eye through the dehydrated sclera. Arteriovenous crossings are present in all areas of the choroid but they are most common posteriorly.

One or more small arteries branch forward from the anterior choroidal arteries, penetrate the sclera, and contribute to the perilimbic vascular arcades.¹³ Sometimes these branches are supplied by the recurrent choroidal arteries before they reach the choroid from the major iridic circle. Here then, is an anterior connection between the intraocular and extraocular vasculature. In man the anterior ciliary arteries, which are not present in the rabbit, represent such a connection but blood flow is in the opposite direction.

The Inner Vascular Layers of the Choroid: Vessels pass centrally from the ciliary arteries from which the afferent arterioles of the choriocapillaris are formed. My observations permit me to agree with Vilstrup's⁸ classification of the terminal arterioles into two broad groups.

The first group consists of fine branches which commonly travel long straight courses while maintaining the same size lumens.

These vessels are substantially narrower than the wide meshes of the choriocapillaris which they form. In the posterior half of the choroid the fine arterioles are abundant, particularly in those areas which lie close to the points of entry of the short ciliary arteries into the choroid. Anteriorly they become scarce. The fine arterioles often form loose anastomosing networks posteriorly, from which the terminal arterioles pass directly to the choriocapillaris. I was unable to identify networks in the anterior regions of the choroid.

The second group is made up of short wide terminal arterioles which usually pass only a short distance from the parent trunk before dispersing in the choriocapillaris. It is difficult to assess whether these wide vessels or the fine arterioles comprise the majority posteriorly, but anteriorly the wide vessels predominate. Posteriorly they almost exclusively reach the choriocapillaris by turning sharply inwards with the result that they enter the membrane nearly at right angles. The same manner of entry may be observed anteriorly but here, the majority of

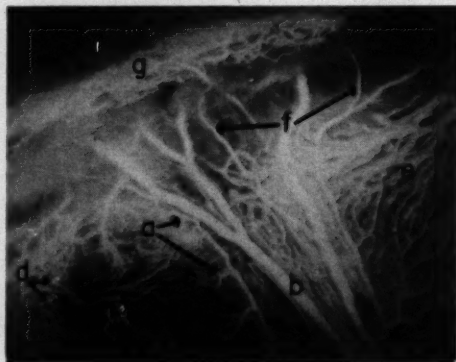


Fig. 7 (Ruskell). External view of the anterior choroidal vessels. The arteries (a) leave a dorsal branch of the medial long ciliary artery (b), break up into capillaries (c), and then form veins (d). In this limited area, above the long ciliary artery, the choroid is composed of a single vessel layer instead of the three layers found elsewhere (e). Two branches (f) from separate anterior choroidal arteries are shown penetrating the sclera to contribute to the perilimbic vessel plexus (g). $\times 11$

the wide terminal arterioles approach the choriocapillaris obliquely. The latter frequently continue into the choriocapillaris, which is a departure from the general pattern. All other arterioles break up immediately upon reaching the choriocapillaris.

The choriocapillaris is composed of a single vessel layer throughout the choroid, the meshes occupying a greater area than the interstices. The meshwork pattern is approximately the same in all regions of the choroid except in a few localized areas. On both sides of the eye, immediately above the intrachoroidal part of the long ciliary arteries, the vascular pattern of the choroid is quite different from elsewhere. With transillumination of the proptosed eye, in which the sclera has been dehydrated, these zones appear avascular as Wudka and Leopold¹ noted. They are vascularized however, but in place of the general triple-layer pattern of the choroid the following is substituted. From two to three anterior choroidal arteries divide to form capillary meshes which drain superiorly into veins, all of these vessels lying in the same plane (fig. 7). Occasionally an early recurrent branch from the major iridic circle contributes to these zones. The zones are long and narrow and extend from the point where the long ciliary artery enters the choroid to the anterior border, where the zones are broadest. The zones vaguely describe a triangle with their bases anterior and hence, Scullica¹⁰ called them the "triangular zones." In some eyes these zones cover a smaller area of the choroid and occasionally they extend below the long ciliary arteries.

In the transilluminated eye other small areas of reduced vascularization may be seen between the upper vortices and again between the lower vortices. These areas are avoided by the large veins of the choroid as a result of their sharp convergence to the vortex centers. A division of the arteries, similar to that of the "triangular zones" is occasionally present in the central parts of these areas.

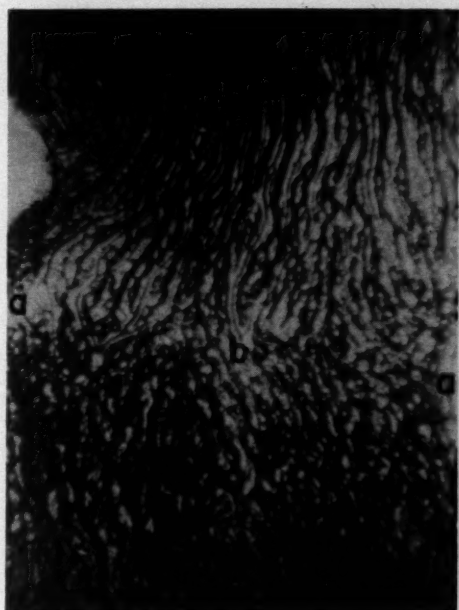


Fig. 8 (Ruskell). The posterior vessels at the horizontal meridian (a, a). Where the arteries (b) enter the choroid the veins are narrowest. The veins increase in lumen as they pass dorsally and ventrally from the venous "divide." The clear space marks the position of the papilla. India ink injection. $\times 10$

The efferent venules of the choriocapillaris travel a short distance within the membrane, draining several capillaries, before turning away obliquely to the adjacent layer of small vessels. Here, they quickly join neighboring venules and the resulting stems usually run a very short course before draining into the nearest large vein. The shortness of the venules partially explains the predominance of arteriole trunks in the middle layer of the choroid, but in fact there are many fewer venules than arterioles.

The Veins: The veins of the choroid are considerably larger than the arteries and, generally, where the arteries are largest the veins are smallest. It follows then, that the veins are smallest along the horizontal meridian, where the short ciliary arteries enter the choroid. Posteriorly this meridian marks what may be described as a "divide" for the

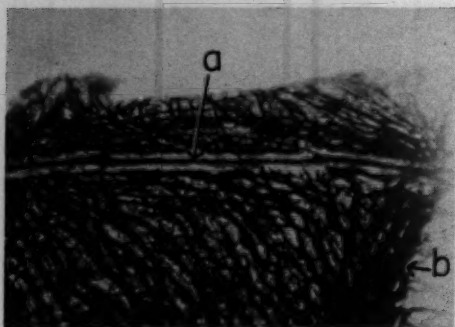


Fig. 9 (Ruskell). The long ciliary artery (a) is not crossed by the choroidal veins. Part of the venous circle of Hovius (b) is seen anteriorly. India ink injection. $\times 13$.

venous blood flow, but the small veins on the opposite sides of the divide are irregularly connected (fig. 8). In this small area it appears that the venous blood flow may pass in either one direction or the other i.e. to the upper or lower half of the choroid. Advancing superiorly or inferiorly towards the vortices, the veins become larger as a result of the many venules draining to them along their course. More anteriorly along the horizontal meridian, where the long ciliary arteries enter the choroid, the veins do not cross from the upper to the lower half of the choroid. The long ciliary arteries are crossed only by the choriocapillaris (fig. 9), and hence the venous "divide" is completed anteriorly.

The veins frequently interanastomose, but the separate identity of any single vein is fully maintained until the vortex center is reached (fig. 4). The number of vortices is variable. Most commonly there are four superiorly and four inferiorly, arranged in pairs. Often, one pair is replaced by a single vortex, and occasionally a smaller vortex is located proximal to these. The vessels which drain the vortices pass obliquely and proximally through the sclera. The veins from pairs of vortices quickly anastomose within or just external to the sclera, and at this stage the vortex veins resolve themselves into four vessels in the majority of eyes. The

subsequent routes of the vortex veins have been described by Prince et al.¹⁴

It is clear that those veins which travel the longest courses, are likely to have the widest lumens. As the vortices are situated anteriorly in the choroid, those vessels passing forward from the posterior pole in the vertical meridian have the greatest distances to travel and they often attain diameters in excess of 400μ . Those veins which lie anterior to the vortices drain most of the blood from the anterior uvea, and although they have only a short course to travel, they are as large as any in the choroid. These anterior veins are relatively few in number, a feature which further explains their large size, and they converge rapidly to the nearby vortices (fig. 10). At the anterior border of the choroid the veins are particularly dense and they form what is often referred to as the venous circle of Hovius.

The posterior veins of the dorsal choroid part as they approach the optic nerve entrance and quickly come together again on the other side. A few small veins pass back through the periphery of the optic nerve foramen and enter the intravaginal space of the optic nerve sheaths. The limited size and number of these veins indicate that this drainage route from the choroid is probably

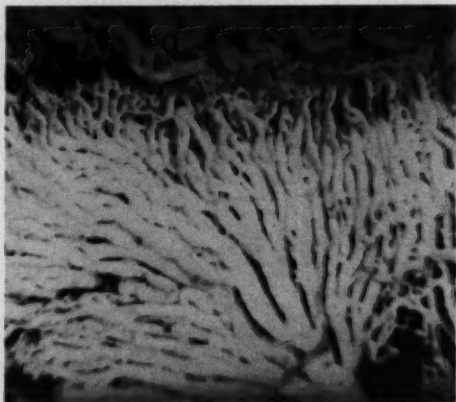


Fig. 10 (Ruskell). The large anterior choroidal veins converge rapidly to the vortex center, a, the major iridic circle. $\times 13$

of little importance. The largest of the veins found in the intravaginal space pass from the retina, not the choroid.

The Vascular Arrangement at the Optic Nerve Entrance: As I stated earlier, from two to four of the short ciliary arteries pass dorsally to penetrate the sclera adjacent to the optic nerve. These arteries anastomose to form an incomplete vascular circle within the sclera, around the optic nerve (fig. 11). The ring is usually complete superiorly but is always broken below the nerve. Occasionally a fine collateral vessel is present, but more often the partial circle is composed of a single vessel from which branches pass principally to the choroid, but others contribute to the pial vascularization. A few fine twigs pass directly into the nerve close to the papilla. These twigs cannot be considered vessels of the lamina cribrosa because this structure is virtually absent in the rabbit.

In most of the animals used in this study there were three arteries passing to the optic nerve area, one of which penetrated the sclera below the nerve (fig. 11). It quickly divided in the choroid, and the branches turned back quite sharply to travel dorsally in keeping with the general direction of blood flow in this region. This inferior vessel seldom connected with the upper vessels. Most branches from these three arteries passed forward in the choroid between the two most medial of the upper vortices, the largest of them terminating only a short distance from the anterior edge of the choroid.

DISCUSSION

The question whether interanastomoses occur between the arteries which nourish the choroid is of some clinical importance and consequently it has frequently been discussed in the literature.¹⁷ The rabbit has been used in experimental work directed to find a comparative answer to this question.

Siegrist⁸ ligated individual ciliary arteries and injected dye into the carotid artery. He observed that the area of dispersion of a

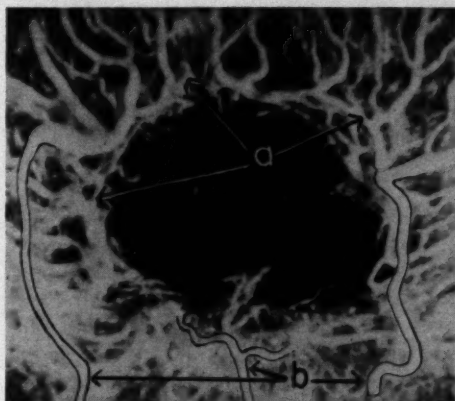


Fig. 11 (Ruskell). The arteries at the entrance of the optic nerve, which has been removed, a, the incomplete arterial circle, b, the three afferent arteries which have been outlined. The inferior artery does not contribute to the "circle." $\times 29$

single ciliary artery within the choroid was not filled from neighboring vascular beds. Earlier, Wagenmann⁴ claimed to have achieved a localized choroidal atrophy in the rabbit after sectioning a few short posterior ciliary arteries and concluded that these vessels must be functional end-arteries. Nicholls⁵ repeated this experiment and was unable to produce localized atrophy until two thirds of the short ciliary arteries had been sectioned, when the eye more often either suffered no degeneration or became phthisical. Most authors of recent years agree with Nicholls that these are not end-arteries in the rabbit,^{6,10,16} and my findings emphatically support this view. That the short posterior ciliary arteries interanastomose behind the eye and within the sclera, is indisputable, although the number of interanastomoses is variable. In the large vessel layer of the choroid anastomoses are again occasionally present but not, I think, to the extent that one may consider that a network is formed, as Scullica reported.¹⁰ However, in the middle layer of the choroid some of the arterioles do form a network in the posterior half of the eye.

In accord with Leber⁷ and Vilstrup,⁸ I do

not consider that connections exist between the recurrent arteries and those of posterior origin except at a capillary level, but this view is not unanimous.¹⁰

There is some disagreement also regarding the existence of arterial interanastomoses in the human choroid but the evidence supporting their presence is convincing.^{16,17}

Scullica^{10,11} and Correia¹⁶ located arterio-venous anastomoses in the rabbit choroid. Both authors found them in the small vessel layer and Scullica also described recurrent arteries passing directly into veins near the vortex centers. Contrasting with these reports is an extensive study by Vilstrup⁸ who was unable to find arterio-venous anastomoses in any part of the choroid. After a careful search of a large number of injected eyes I have found only two arterio-venous anastomoses (fig. 6), both involving the recurrent arteries and similar to those found by Scullica. It appears likely that arterio-venous anastomoses, although present in the rabbit choroid, are quite rare, and little importance can be attached to their role as bloodflow regulators.

Several investigators^{18,19,20} have reported evidence of arterio-venous anastomoses in the human uvea, but some of this work has been firmly criticized.^{21,22}

A local arterial bloodflow regulator mechanism in the eye of the rabbit involving the choroidal vasculature, has been postulated by Sautter and Seitz.²³ The effectiveness of their experimental arrangement depended in part, upon a strict similarity between the anatomy of the human and rabbit vasculatures, which is not the case.²⁴ However, the optic nerve and retinal vascularization were primarily involved here and I shall therefore make no further comment.

Most writers refer to a circle of Zinn (circle of Haller) in the rabbit, similar to that of man. My observations show that the vascular arrangement in the optic nerve region of the choroid is significantly different from that of man. The majority of the short posterior ciliary arteries in man, penetrate

the sclera close to the optic nerve. This is not the case in the rabbit, in which most of these vessels enter the eye well below the optic nerve. It is therefore hardly surprising to find that a complete circle of Zinn is not formed. It has been seen that the partial arterial circle which is formed, is usually composed of a single fine vessel, whereas the complete circle of man is relatively large and frequently double. The choroidal branches from the "circle" all pass dorsally, and not in all directions as in man. Branches to the optic nerve, immediately behind the papilla, and to the pial sheath, are common to man and rabbit.

The inner vascular layers of the choroid have been fully described by Vilstrup⁸ and my findings are largely in accord with hers. Virchow⁶ found that some terminal arterioles continued into the choriocapillaris but Vilstrup claimed that they all terminate at this membrane. On this point I am in agreement with Virchow, but in the posterior half of the choroid the arterioles are nearly all as Vilstrup described them.

SUMMARY

Neoprene latex casts of the ocular blood vessels were prepared and the anatomy of the choroidal vessels was examined.

The whole of the choroidal vasculature is described in some detail. A careful search was made for arterial interanastomoses and arterio-venous anastomoses. The former were found quite frequently between the short posterior ciliary arteries before these enter the choroid, and less commonly in the outer layer of the choroid. In the layer of small vessels, irregular networks were formed by some of the arterioles posteriorly.

The recurrent choroidal arteries usually break up into capillaries, but in two eyes I found a direct connection between a recurrent artery and a large vein near the vortex center. No further arterio-venous anastomoses could be identified.

There is little junction between the venous bloodflow of the dorsal and ventral choroid,

but otherwise there is a considerable inter-connection between the veins.

A complete arterial circle is not formed within the sclera about the optic nerve in the rabbit. All specimens showed a fine partial circle, complete dorsally but broken ventrally. The vessels passing from the "circle"

largely correspond with those of the circle of Zinn in man.

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THE BEHAVIOR OF TUMOR CELLS FROM THE WALKER CARCINOSARCOMA 256 IN THE OCULAR TISSUES OF RATS*

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This report deals with the production of malignant tumors in animal eyes and their transmission to the eyes of, homologous hosts, through donor material contaminated with cancer cells. The incidence of cancer cells in the blood of animals suffering from malignant diseases of the eye and orbit are also presented. This is part of a larger investigation of human donor eyes obtained from patients dying of various causes and of animal eyes in which certain diseases have been produced.

Walker carcinosarcoma 256 is a highly malignant tumor in certain strains of rats. In susceptible hosts it can be transferred by intramuscular and subcutaneous injection of tumor fragments. The transplanted tumors grow rapidly and kill the animal in five to six weeks. Histologically the growth consists of mixed types of cells. The two architectural patterns, carcinoma and sarcoma can occur separately or can form a mixed type of growth.¹ We are not aware of other work on the reproduction of this tumor in the eye or orbital tissues.²

METHODS AND MATERIALS

Tumor cells, suspended in saline and antibiotics were supplied by Dr. J. Salter of the Banting and Best Medical Research Department, University of Toronto. These cells were obtained from a Walker carcinosarcoma 256 which was grown in the muscle tissue of a Wistar rat.

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Sixty male Wistar rats, each weighing about 200 grams, were divided into three equal groups. Into each animal of Group I, a suspension containing 150 cells in one cc was injected intramuscularly, in the gluteal region. Into each animal in Group II a suspension containing 150 cells in 0.2 cc was injected into the vitreous chamber, through the sclera, of the right eye. Each animal belonging to Group III received, into the right orbit, 150 cells in 0.2 cc of the latter suspension.

Each group was then equally divided into two sub-groups A and B. The animals in sub-group A were sacrificed on the eighth day, those in sub-group B on the 21st day from the date injection. Before the rats were sacrificed samples of blood were taken from each animal, to study the incidence of free cancer cells in the blood.³

Immediately after the death of an animal the tumor bearing tissue was removed. Smears were made from the cut surface of the tumor and were stained by the Papanicolaou method. Paraffin sections were prepared and stained with haematoxylin and eosin. Ocular tissues, orbital contents, brain and meninges, lungs, lymph nodes and other tissues were removed for histopathological studies. A new tumor cell suspension was made from the intraocular growth. A new series of 10 male Wistar rats was injected intraocularly with 150 cells in 0.2 cc of the suspension. From this generation of ocular tumors two further generations were produced, each in 10 homologous animals.

Smears of the ocular fluids and paraffin sections of the tissues from most of the apparently normal eyes of the cancerous animals were studied for malignant cells. These animals had intramuscular tumors as well as

growths in the opposite eye and orbit. To test for any cancer producing factor(s) in these apparently normal eyes, cell suspensions were made from some of the eyes of rats sacrificed on the eighth day. From each suspension 0.2 cc was injected into one eye of five normal rats.

A cell-free filtrate of the original tumor cell suspension was injected: one cc intramuscularly into five normal rats and 0.2 cc into the eye and orbital tissues of another two sets of five normal rats. This experiment was done to test if the cell-free filtrate would produce malignant growths.

RESULTS

In every one of the original 60 animals who received 150 tumor cells, a tumor was visible after a latent period of five to six days. Suspensions of 75 cells per cc and the cell-free filtrates in no instance produced a tumor.

In Group I, the intramuscular tumors became visible in a week, progressed rapidly and by three weeks were about two inches in diameter. They contained hemorrhagic spots and showed no sign of capsulation. Their cells were typical of Walker carcinosarcoma.

In Group II, the intraocular tumors were visible in a weeks time (fig. 1). They grew rapidly and ruptured the globe, usually at the limbic region. In three weeks each tumor attained one inch in diameter. The tumor contained hemorrhagic areas and inflammatory exudate. There was no evidence of capsule formation. Smears and sections demonstrated the characteristics of Walker Carcinosarcoma. Masses of tumor cells filled the vitreous cavity and the subretinal space (fig. 2). Many of the ocular structures including the lumia of blood vessels, were invaded by cancer cells (fig. 3). In a number of sections the adjoining orbital tissues were infiltrated.

In Group III, the intraorbital tumor grew rapidly, displacing and distorting the eyeball. In three weeks the tumors attained about one and one half inches in diameter.



Fig. 1 (Basu, Sibay, and Chang). Intraocular tumor, Group II, 10 days after the injection of a tumor cell suspension into the eye.

At this stage, it was almost impossible to identify the eye within the tumor mass. The growth was diffuse and was covered with hemorrhagic areas and a large amount of inflammatory exudate. Smears and sections showed typical malignant cells. In some sections the tumor cells invaded the ocular structures which were extremely disorganized. The orbital bones and the lacrimal gland also were infiltrated.

Attempts to transplant tumors from the eye of one animal to that of another were uniformly successful. The tumors grew rapidly. Successive generations retained the cellular characteristics of the original tumor.

Cellular suspensions from the macroscopically normal eyes of the animals which were sacrificed on the eighth day did not transmit the growth to other animals. Smears and sections made from these eyes were free of atypical cells.

Other tissues, as brain, meninges and

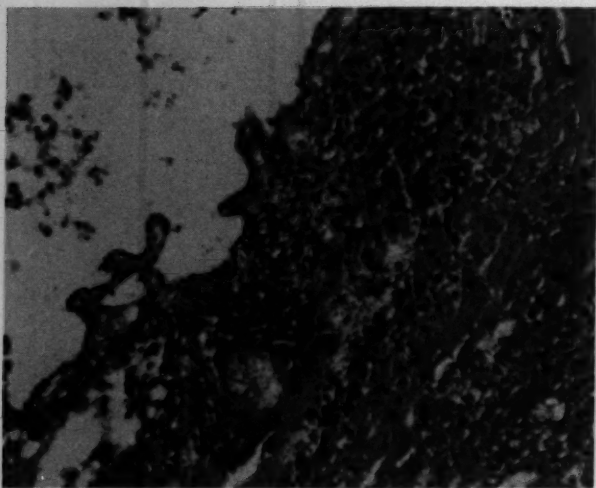


Fig. 2 (Basu, Sibay, and Chang). Tumor cells in the uveal tissues and vitreous, Group II (Hematoxylin-eosin, $\times 225$).

lungs, of the animals belonging to all of the three groups which were sacrificed on the eighth day were free of metastases. On the other hand, tissues from the animals sacrificed on the 21st day showed cancer cells in most of the organs (figs. 4, 5, 6 and 7). Blood from the animals which were sacrificed on the eighth day were free of malignant cells. However, free cancer cells were

found in the blood of the rats which were sacrificed on the 21st day. Malignant cells were present in the blood of four out of ten animals in Group I and in all of the animals in Group II and III (fig. 8).

DISCUSSION

In human ocular surgery, material obtained from the eyes of persons dying of

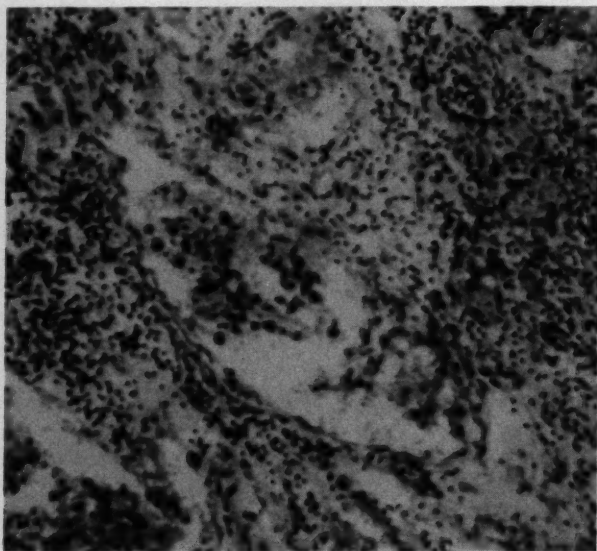


Fig. 3 (Basu, Sibay, and Chang). Tumor cells in the lumen of an ocular blood vessel, Group II (Hematoxylin-eosin, $\times 225$).

Fig. 4 (Basu, Sibay, and Chang).
Tumor cells in the lung, Group II
(Hematoxylin-eosin, $\times 225$).

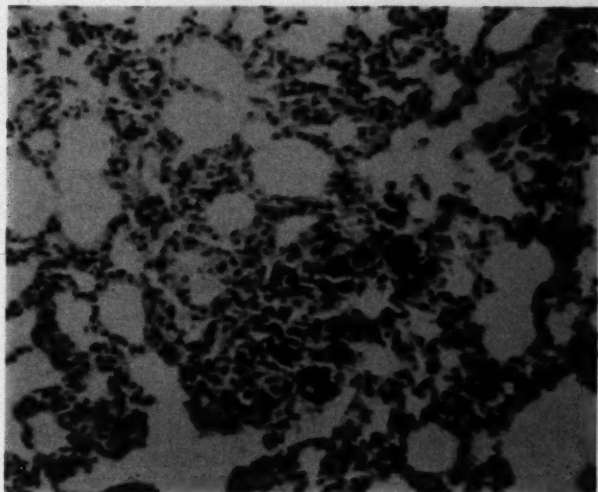
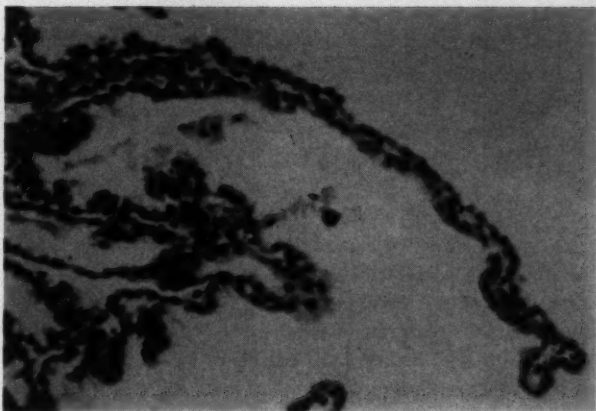


Fig. 5 (Basu, Sibay, and Chang).
Tumor cells in the pia mater,
Group II (Hematoxylin-eosin, $\times 225$).

Fig. 6 (Basu, Sibay, and Chang).
A single tumor cell on the choroid
plexus of the 4th ventricle, Group
II (Hematoxylin, $\times 225$).



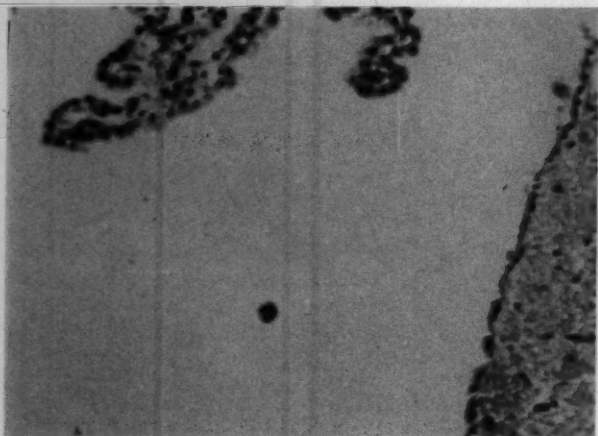


Fig. 7 (Basu, Sibay, and Chang). A free tumor cell in the 4th ventricle, Group II (Hematoxylin-eosin, $\times 225$).

cancer other than ocular cancer, is commonly used for transplantation. There is some difference of opinion regarding the use of donor material obtained from an eye containing a malignant growth. The case of Hata's is the only clinical report of transplantation of a malignant tumor by means of ocular donor material.⁴ In this case a glioma of the retina was transplanted by means of a corneal graft.

In the present study it has been possible to reproduce Walker carcinosarcoma in the

ocular and orbital tissues of rats. This type of intraocular malignant growth can be transmitted to the eyes of other homologous hosts by a suspension contaminated with cancer cells. Possibly Walker carcinosarcoma in rats is not analogous to human cancer and the genetic variation in humans is wider than in rats. However, one may still wonder whether it is really safe in human ocular surgery to use donor material from cancerous individuals in general and particularly from eyes containing malignant

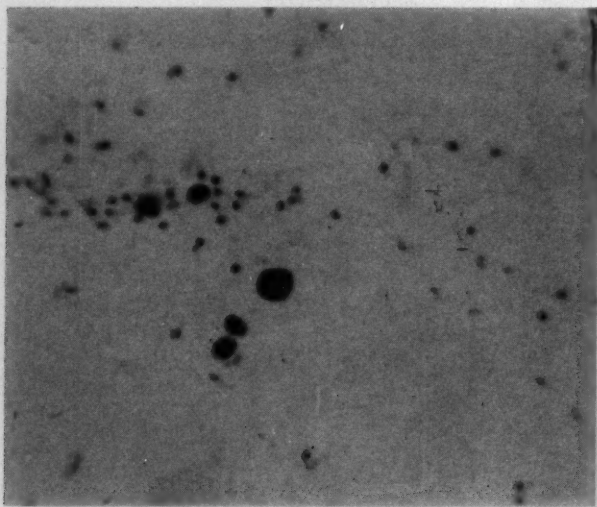


Fig. 8 (Basu, Sibay, and Chang). Free cancer cells in the blood, Group II (Papanicolaou stain, $\times 450$).

growths. How can one be sure that these materials are free from carcinogenic factors.

The presence of cancer cells in the blood of all of the rats having advanced intraocular and intraorbital malignant growths is interesting. Studies of cancer cells in the blood of patients suspected of having intraocular or intraorbital growths could be of diagnostic value.

SUMMARY

Suspensions of tumor cells from Walker Carcinoma were injected into the gluteal muscle, vitreous chamber and orbital tissues of Wistar rats. In every one of these animals who received 150⁶ cells, a tumor was visible after a latent period of five to six

days. Suspensions containing 75 cells per cc and the cell-free filtrates in no instance produced a tumor. The tumors showed the characteristics of Walker Carcinoma. Attempts to transplant tumors from the eye of one to that of another were uniformly successful. Successive tumor cell generations retained the cellular characteristics of the original tumor. Metastases from advanced intraocular and intraorbital growths were observed in the lungs, brain, meninges and other tissues. Free cancer cells were detected in the ventricles of the brain. Free cancer cells were found in the blood of all rats having advanced intraocular and intraorbital growths.

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REGULATORY CHANGES IN THE DYNAMICS OF INTRAOCULAR PRESSURE EVOKED BY TONOMETRY AND TONOGRAPHY*

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The concept that active regulation is responsible for the maintenance of a constant level of intraocular pressure in the normal eye is attracting greater attention than ever before. In the experimental animal, changes in the intraocular pressure are associated with parallel changes in the activity along certain sensory nerves;^{1,5} furthermore, the intraocular pressure, as well as, the dynamics

of the steady state may be altered by varying experimentally the activity of certain peripheral^{1,10,12} and central^{2,6,16} components of the nervous system. These findings have enhanced the expectation that regulation is achieved via the nervous system.

In the human, the procedure of investigating the dynamics of intraocular pressure by tonography⁷ entails a significant provocation of the constancy of the intraocular pressure level; the intraocular pressure is raised and maintained high by the tonometer load throughout the procedure. Yet, there has been no evidence that regulatory changes in

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the dynamics occur during the procedure; nor has it been found necessary to abandon the assumption on which the validity of this technique rests, namely, that the dynamics of the steady state that existed before tonography were not altered but remained the same throughout the procedure.

Single tonometry in the human has been shown to produce a significant reduction in tonometric readings that are obtained on the same eye thirty seconds⁴ or one minute later;¹⁴ and a greater reduction in the reading obtained after four minutes.¹⁴ Tonography on one eye has been shown to reduce the pressure in the other eye.¹¹ A systematic error in the calibration tables which differs significantly for different parts of the Schiøtz scale has been reported.⁵ A rise in pressure has been shown to reduce the inflow rate in the experimental animal⁸ and increase the resistance to outflow in the excised eye.³ In the calculation of tonographic values the average value of E , P_v and ΔP_v is used.^{7,9} These various considerations reduce, markedly, the sensitivity of tonography to regulatory changes occurring during the procedure and confuse the correspondence between the calculated values and the parameter they propose to represent. However, the calculated values have not been shown to contradict the assumption.

It is the object of this communication to show that when the results of tonography are analyzed with regulation in view, the assumption that the dynamics of the steady state are not affected by tonometry or tonography is not tenable.

MATERIAL AND METHOD

THE SAMPLE

For this study, 291 subjects between the ages of 40 and 70 years were selected at random to satisfy the following requirements:

1. Absence of ocular complaints and of history of ocular disease or surgery.
2. Corrected visual acuity of 20/20 or better as tested by the Snellen's chart.

3. Normal central visual field with the 1/1000 white target.

4. Absence of evidence, in the slit-lamp examination, of present or past sclero-corneal damage that might interfere with the measurements.

5. Ability to tolerate the procedures to tonometry and tonography comfortably without recourse to the external support of the lids.

6. During tonometry and throughout the entire tonogram, the scale reading of the 5.5 Gm. plunger load of the Schiøtz tonometer is to remain within the range of 3 to 10 units.

PROCEDURE

Adequate anaesthesia was achieved by the topical application of 0.5 percent Ophthalmic solution of Proparacaine to the cornea and the conjunctival sac. The procedure of testing was adequately and reassuringly explained to each subject. Next, the Mueller electronic tonometer, coupled to a Leeds and Northrup Speedomax Recorder and kept in continuous operation, was calibrated against the micrometer.⁴ Then, for ten or fifteen seconds, the tonometer was held slightly above the cornea of the eye to be tested first, before it was lowered to be completely and uniformly supported by the cornea. It was kept in this position for ten to fifteen seconds to provide a record of the tonometric reading. In a similar manner, tonometry was performed on the second eye. Then, the tonometer was again held for fifteen seconds above the cornea of the first eye before it was lowered for the purpose of obtaining a four-minute tonogram. In a similar manner, tonography was then performed on the second eye.

The sequence of testing was changed regularly to allow equal chances for right and left eyes to be tested first. Thus, in one case, the sequence was: tonometry O. D., tonometry O. S., tonography O. D., tonography O. S., and in the next case O. S. preceded O. D.

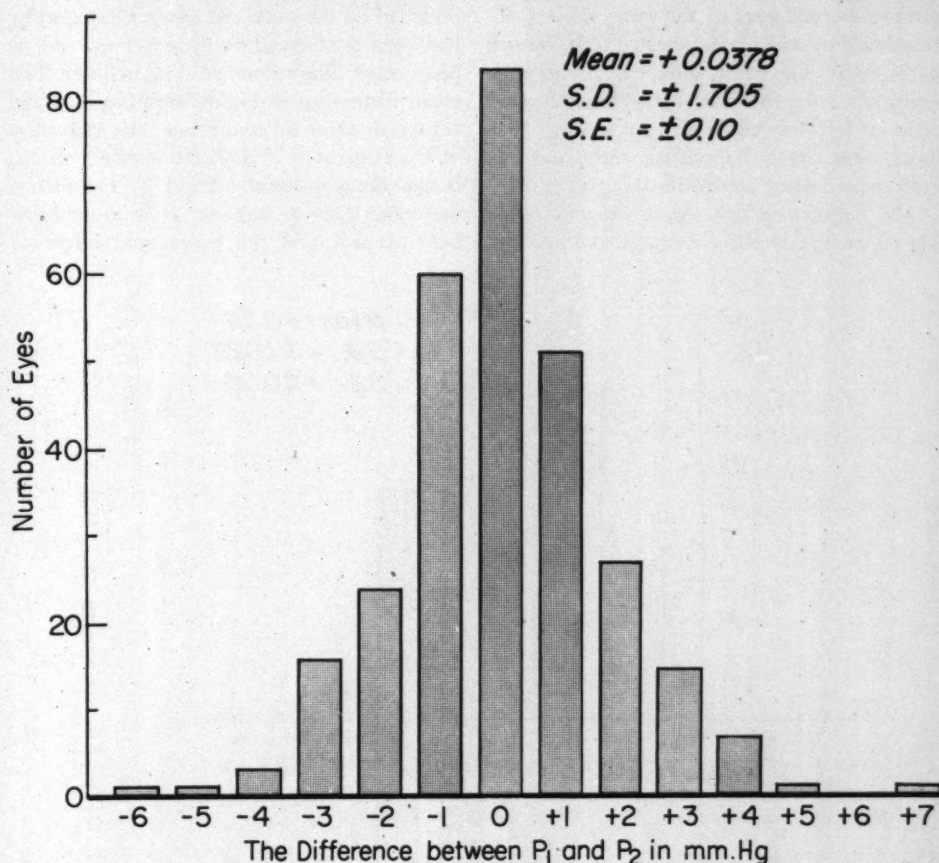


Fig. 1

The 1955 calibration tables⁹ were used to calculate the pressure readings and the C-values of the tonogram. F-values were calculated from the equation:

$$F = (P_0 - 10) \cdot C$$

where in, F represents the inflow rate of aqueous in cu. mm/min., P_0 , the pressure reading at the beginning of the tonogram, in mm Hg, C the facility of outflow in cu. mm/mm Hg/min. and 10 is the average episcleral venous pressure.

RESULTS AND INTERPRETATION[^]

P_1 and P_2 will represent the pressure readings of the initial tonometry on the first

and on the second eye respectively. P_{01} and P_{02} will represent the pressure reading at the beginning of the tonograms, C_1 , C_2 and F_1 , F_2 will represent the tonographic values calculated for the first and for the second eye respectively.

1. ANALYSIS OF PRESSURE READINGS P_1 , P_2 , P_{01} AND P_{02}

The frequency distributions of the differences $P_1 - P_2$, $P_1 - P_{01}$, $P_2 - P_{02}$ and their respective statistics appear in Figures 1-3 and Table 1.

The hypothesis that no difference exists between the tonometric readings obtained on

the two normal eyes of the same subject, irrespective of which eye is tested first, cannot be rejected. The mean difference is not significantly different from zero. The effect of right or left was eliminated by the fact that there were equal chances for right and left eyes to be first or second in the procedure.

The hypothesis that the tonometric reading on an eye is not different from another

obtained on the same eye, after a tonography had been performed on the other eye had to be rejected. The second reading is lower. The mean difference is highly significant at the 1 percent level of confidence; the reduction in P_{02} is greater. Whether this effect on P_{02} is the same ipsilateral effect of tonometry, becoming greater in time, or is a combination of that and the consensual effect of

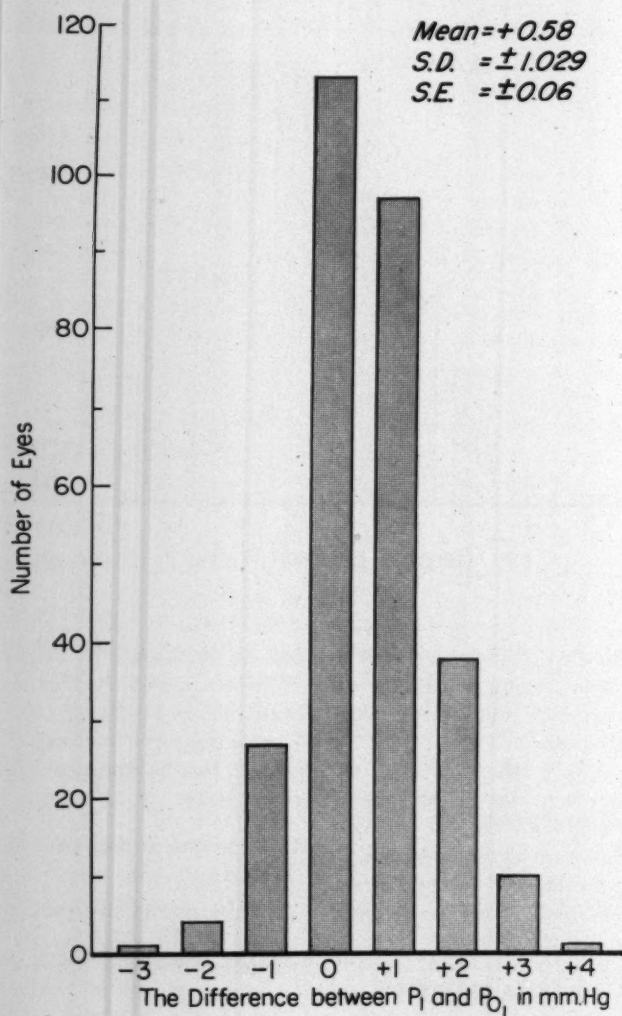


Fig. 2

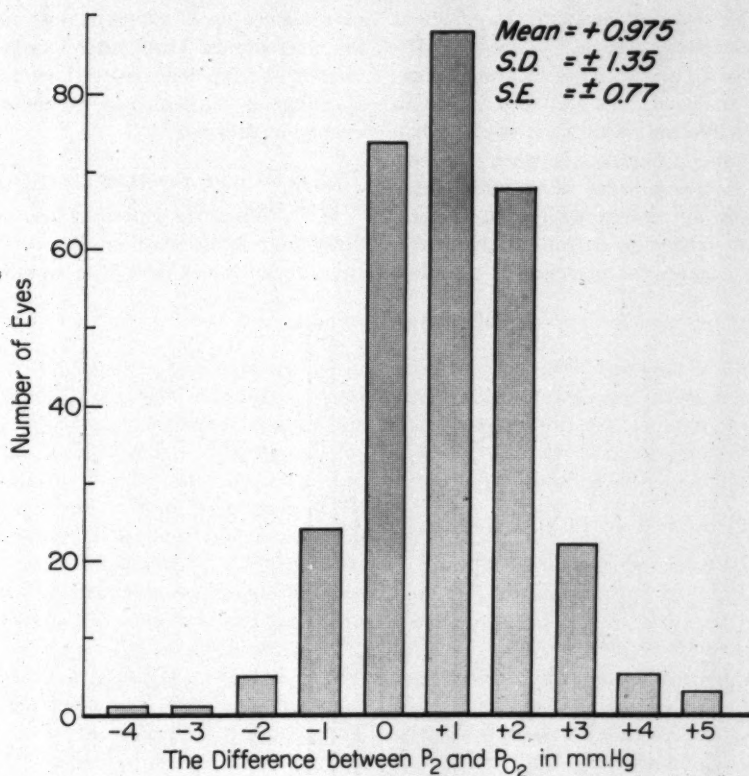


Fig. 3

tonography cannot be discerned in this design. Both effects have been reported.^{11, 13, 14}

The significant feature of the distribution of the difference $P_2 - P_{02}$ is the finding that P_{02} was not always lower than P_2 : It was equal to in 25 percent, higher in 11 percent and only one mm lower in 30 percent. This is of critical importance for the search for regulatory changes in the dynamics of intraocular pressure; such changes cannot be considered independently of the change in pressure they are to regulate.

The question of whether there are two effects, a rise and a reduction of P_{02} , instead of one is difficult to answer in the absence of information regarding the reproducibility of the tonometric reading under similar conditions, when the intraocular pressure is kept

constant. The relative frequency of the rise was not related to the pressure level. The magnitude of the reduction increased only slightly at higher pressure levels.

2. ANALYSIS OF TONOGRAPHIC VALUES

The frequency distribution of all C-values and its statistics appear in Figure 4 and

TABLE 1

Distribution	Mean	Standard deviation	Standard deviation of the Mean
$P_1 - P_2$	+0.0378	1.705	0.10
$P_1 - P_{01}$, O.D. 1st	+0.616	0.94	0.076
O.S. 1st	+0.542	1.16	0.098
Total	+0.58	1.029	0.060
$P_2 - P_{02}$	+0.975	1.315	0.077

Table 2. In order to evaluate the effect of sequence of tonography on C-values, the distributions of C_1 and C_2 were separately compiled and analyzed. The statistics appear in Table 2. While the mean C_2 is smaller than mean C_1 , the difference between the two means is not significant statistically. This finding is in agreement with what was reported by other investigators. However, while this negates the presence of an effect

of sequence on C values it does not negate the presence of regulatory changes in C_2 -values. For this purpose we have to rule out a change in C_2 which is regulatory to the change in pressure.

THE EFFECT OF PRESSURE CHANGE ON C_2

If C_2 -values are separated into subgroups depending upon whether P_{02} was greater, equal to, or lower than P_2 a significant reg-

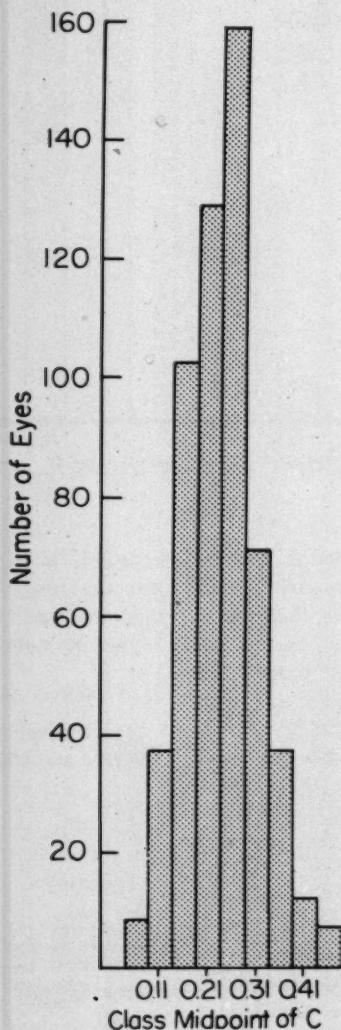


Fig. 4

TABLE 2
THE STATISTICS OF C-VALUES

	Mean C	Standard deviation	Standard deviation of the Mean
For All Tonograms	0.239	0.076	0.003
For First Tonograms	0.245	0.078	0.004
For Second Tonograms	0.233	0.074	0.004

TABLE 3
THE CHANGE IN C_2 AS RELATED TO THE CHANGE FROM P_2 TO P_0

	Mean C	Standard deviation of the Mean
All First Tonograms	0.245	0.004
From Second Tonograms		
When $P_2 < P_0$	0.26	0.015
$P_2 = P_0$	0.243	0.007
$P_2 - P_0 = 1$	0.228	0.007
$P_2 - P_0 = 2$	0.21	0.007

ulatory relationship becomes evident, Table 3. The mean C_2 in these subgroups varies inversely with the direction of the change from P_2 to P_0 . The statistical significance of this relationship may be tested by comparing the means of the various subgroups of C_2 with each other, with the grand mean of C_2 or with the grand mean of C_1 . All these tests show that the reduction in C_2 in the subgroup wherein P_0 was two or more mm lower than P_2 is highly significant at the 1 percent level of confidence.

Thus, the hypothesis that C_2 values are independent of the effect of the preceding events i.e. tonometry on the same eye and tonography on the other eye, is not tenable. A regulatory relationship is manifest: the C_2 values are significantly reduced when the

preceding events produced a reduction 2 mm Hg or more in the pressure reading. It follows that from the standpoint of clinical prediction the predictive content of a low C_2 value is not identical with a similar C_1 value.

THE EFFECT OF P_0 ON C_1 AND C_2 VALUES

Since a regulatory process may in addition to pressure change, be related also to the P_0 level, C_1 and C_2 were separated into groups of different P_0 levels and with different direction of pressure change from P to P_0 , Table 4. The results show that when tonometry produced no effect on the P_0 reading ($P_1 = P_{01}$). The mean C_1 values for different P_0 levels were not significantly different from each other at the 1 percent level of confidence. On the other hand, wherever

TABLE 4
THE CHANGE IN C-VALUES FOR DIFFERENT P_0 -LEVELS WITH THE CHANGE FROM P TO P_0
 P_0 Reading

	9-13		14-18		19-23	
	Mean C	Standard Deviation of the Mean	Mean C	Standard Deviation of the Mean	Mean C	Standard Deviation of the Mean
All C_1	0.212	0.004	0.266	0.005	0.245	0.008
C_1 when						
$P_1 = P_{01}$	0.23	0.0095	0.254	0.009	0.23	0.016
$P_1 - P_{01} = 1$	0.175	0.009	0.244	0.011	0.246	0.019
$P - P_{01} = 2$	0.185	0.015	0.258	0.013	0.28	0.021
C_2 when						
$P_2 = P_{02}$	0.223	0.012	0.263	0.012	0.225	0.018
$P_2 - P_{02} = 2$	0.21	0.008	0.222	0.007	0.168	0.002

P_{01} was lower than P_1 , the mean C_1 value for the low P_0 group was markedly reduced. This reduction is statistically highly significant at the 1 percent level of confidence when compared with the means of different P_0 groups that had a similar change from P_1 to P_{01} or with the same P_0 group when P_1 was equal to P_{01} . Thus, the hypothesis that C_1 values are independent of the preceding tonometry on the first eye is not tenable. A significant reduction in C_1 values occurs in the low P_0 groups whenever the P_{01} is lower than P_1 .

When the C_2 values showing significant reduction were analyzed with respect to P_0 level a reduction significant at the 1 percent level of confidence was found in the group with high P_0 level.

THE EFFECTS OF SEQUENCE AND PRESSURE CHANGE ON F VALUES

When F values were treated in a manner similar to that of C values a significant reduction in F_2 values was detected. When F_1 and F_2 were investigated in groups of different P_0 readings, they were not found to differ significantly for the same group of P_0

readings except in eyes wherein P_{02} was lower than P_2 ; in these the F_2 values were significantly reduced for the same P_0 reading Figure 5.

THE EFFECT OF THE SELECTION OF THE STEADY STATE PRESSURE

The preceding analysis has assumed that the steady state pressure is that measured at the beginning of the tonogram. This pressure reading was shown to differ significantly from that of the initial tonometry. The absolute value of C will be significantly influenced by which of these two sets of pressure readings is selected to represent the steady state pressure. Of greater significance is the possible effect of this selection on the regulatory tendency uncovered between C_2 and the change from P_1 to P_0 in the preceding analysis.

In order to evaluate this effect, the following assumptions have to be made:

1. That the steady state pressure is that of the initial tonometry, i.e. P_1 and P_2 .
2. That tonometry does not alter the dynamics of the steady state nor does tonography; the reduction from P to P_0 read-

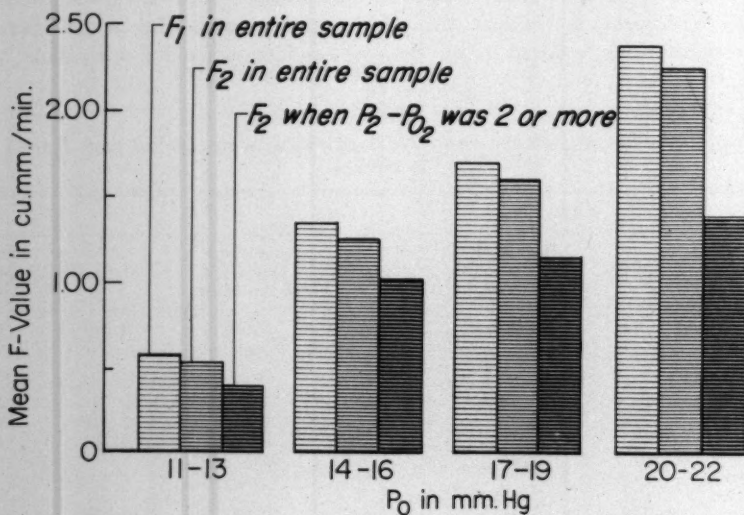


Fig. 5

ings being due exclusively to the increased outflow rate during the procedure. These assumptions enable us to correct the C values by replacing P_0 in the equation

$$C = \frac{\Delta V}{T(Av \cdot P_t - P_0 - 1.25)}$$

by P_1 and P_2 .

C_1 and C_2 values in eyes wherein P_0 was lower than P by two or more mm Hg were recalculated individually by replacing P_0 by P in the above equation. The statistics of the distributions of the corrected C_1 and C_2 appear in Table 5. It is evident that this correction did not eliminate the difference between C_1 and C_2 . The difference between the two means is statistically highly significant at the 1 percent level of confidence. Furthermore, this correction did not eliminate the difference between the C values obtained on eyes in which the P_0 was reduced and those where the P_0 remained equal to P . Those findings indicate that the above assumptions asserting that the dynamics of the steady state are not influenced by tonometry or tonography are not tenable.

THE EFFECT OF THE INSTABILITY OF THE STEADY STATE

Since the dynamics of the steady state were altered during the first, as well as, the second tonogram, the question arises as to whether the meaningful regulatory behavior of C and C_2 may not be due to the difference in the direction of the instability of the steady states that prevailed during each test. A suppression of inflow which is still active during the first tonogram, will increase the calculated C value; if this has ceased or was reversed during the second tonogram, it will reduce the calculated C value. While this uncertainty emphasizes the need for independent indices with which to gauge the steady state or the direction of its instability, it does not explain the findings. For, such an effect should have produced an increase in all C_1 values irrespective of the P_0 reading. Instead, C_1 was found to behave differently

TABLE 5
C-VALUES CORRECTED FOR STEADY STATE P
WHEN $P - P_0 = 2$

	Mean C	Standard Deviation of the Mean
C_1	0.335	0.012
C_2	0.270	0.010

with different P_0 levels. In fact the only change which was statistically significant was not an increase but a reduction in C_1 values when the P_0 reading was low. The same discrepancy holds true for the attempt to explain the reduction in C_2 values by a reversal of this effect.

Thus, a simple effect on inflow rate alone, influencing artificially the calculated C value cannot account for these findings. A regulatory and real change in the resistance to outflow has to be included in the interpretation of the results.

COMMENTS

The findings of this study indicate that significant regulatory changes in the dynamics of the intraocular fluid are evoked by the procedures of tonometry and tonography: A suppression of inflow and a compensatory increase in resistance to outflow. The main positive support for the assumption that these procedures do not alter the dynamics of the steady state that existed before them has been the finding that when tonography is prolonged, P_t (intraocular pressure during tonometry) becomes equal to P_0 (intraocular pressure before tonometry or tonography).¹⁷ In view of the regulatory nature of the change in the dynamics of the steady state, this phenomenon may indeed be the result of perfect regulation.

In tonography, the derivation of C assumes that the inflow rate as well as outflow facility of aqueous were not altered by the procedure. F is calculated by assuming that outflow facility was not altered and that the episcleral venous pressure in each case was 10 mm Hg. These limitations render tonography, by design, incapable of detecting

changes in either one of the dynamics of the steady state that may occur during the procedure and much more so with regards to changes that are of a regulatory nature. Our knowledge of the regulatory process and of the various events that occur during tonography, as well as, the definitive interpretation of the various segments of the tonographic tracing is critically dependent upon our ability to monitor the various parameters of the steady state independently of each other.

From the clinical standpoint these findings emphasize the following:

1. That tonometry, preceding tonography, alters significantly the dynamics of the steady state. This procedure either should be discontinued or the time lapse between it and tonography should be sufficiently long for the eye to recover from this effect. Such modification should reduce the incidence of low C values in normal eyes.

2. In normal eyes, low C values are significantly more frequent in second tonograms

because of the regulatory effect. A better separation between glaucoma and normal may be expected if only C values of the first tonograms are used. A low C value of a first tonogram is more significant in this respect than that of a second tonogram.

3. Because of the significant relationship between P_0 and C, clinical prediction may be made more accurate if the significance of a C value is sought in the P_0 group to which the eye belongs rather than in the entire distribution of C values.

SUMMARY

The effect of Tonometry and Tonography on the intraocular pressure and on the dynamics of the study state was reported in the normal eye. The findings indicated that these procedures alter significantly the dynamics of the steady state that prevailed before their use, and that these changes are regulatory in nature.

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THE RELATIONSHIP OF STEROID THERAPY AND CATARACTS IN PATIENTS WITH RHEUMATOID ARTHRITIS*

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A recent report by Black, Oglesby, von Sallmann and Bunim¹ citing the incidence of posterior subcapsular cataracts (P.S.C.) in rheumatoid arthritis patients treated with steroids has caused much concern. They showed that of 44 patients with rheumatoid arthritis, treated with steroids, 17 (39 percent) developed posterior subcapsular cataracts. These cataracts were observed only in

those patients who had received moderate to high dosages of steroids for periods of one year or longer. The dosage and duration were directly proportional to the incidence of P.S.C. The majority of the patients were between 30 and 60 years of age. Only six of the 17 patients with P.S.C. had subjective visual complaints and all had normal corrected vision. Nineteen controls showed no P.S.C.

In an attempt to substantiate this work, the following study was undertaken.

*From the Department of Ophthalmology, Ohio State University.

AGE and SEX DISTRIBUTION

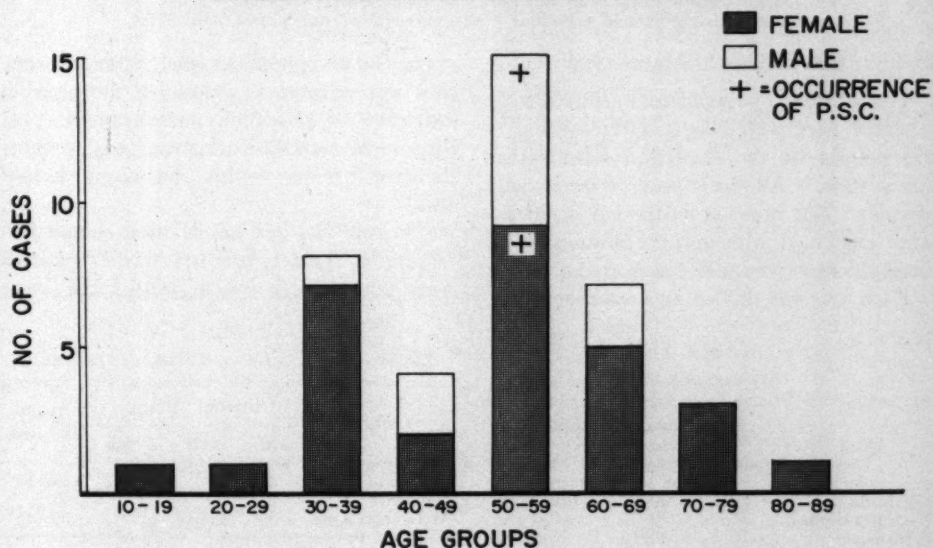
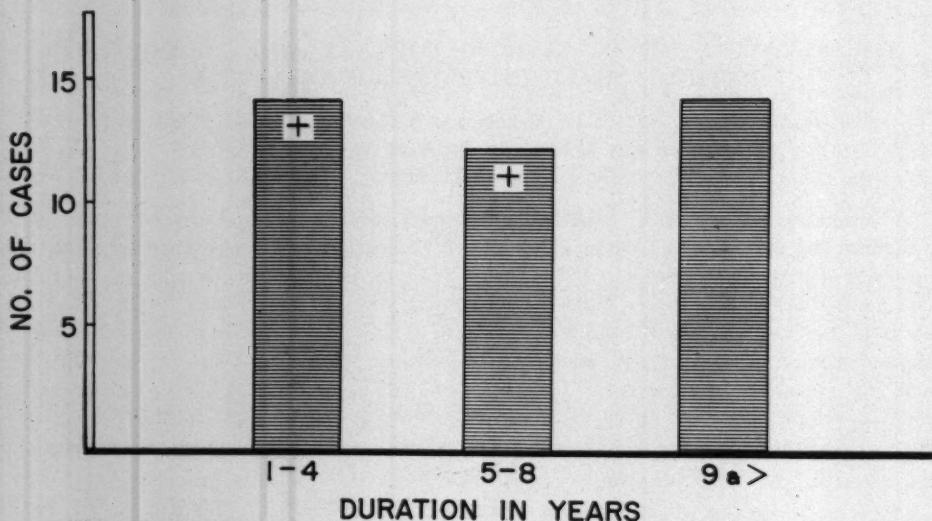


Fig. 1 (Pfahl, Makley, Rothermich and McCoy). The relationship of steroid therapy and cataracts in patients with rheumatoid arthritis. + = occurrence of one patient with P.S.C.

DURATION OF RHEUMATOID ARTHRITIS



+ = occurrence
of P.S.C.

Fig. 2 (Pfahl, Makley, Rothermich and McCoy). The relationship of steroid therapy and cataracts in patients with rheumatoid arthritis. + = occurrence of one patient with P.S.C.

METHODS AND MATERIALS

The 40 cases of rheumatoid arthritis to be reported here were diagnosed according to the criteria of the American Rheumatism Association.² All had been treated with steroids. No non-steroid-treated arthritics were examined, although the desirability of such a control series is recognized.

Each eye was dilated and examined thor-

oughly by an ophthalmologist. Specific attention was devoted to determine the possible existence of conditions which might predispose to cataract formation (e.g., uveitis, diabetes, trauma, toxins and atopic dermatitis).

The age and sex distribution is noted in Figure 1. Twenty-nine of the 40 patients were female. Both patients with P.S.C. were

TABLE 1
STEROID DOSAGE

Drug	Daily Dosage in mg/day		
	low	mod.	high
Cortisone	50	50-99	100 & >
Hydrocortisone	20	20-39	40 & >
Prednisone	10	10-15	16 & >
Dexamethasone	1.6	1.6-29	3.0 & >
Triamcinolone	4	4-14	15 & >
Other analogs	Equiv.	Equiv.	Equiv.

TABLE 2
INCIDENCE OF P.S.C. WITH STEROID TREATMENT

Rx	Steroid Dosage			Total Patients
	low	mod.	high	
Less than 1 yr.	2	1	3 (1)*	6 (1)
1-4 years	9	10	1	20
More than 4 yrs.	3	11 (1)	X	14 (1)
P.S.C. occurrence	14	22 (1)	4 (1)	

* No. in parenthesis indicates case of P.S.C.
X—Indicates no cases available.

in the 50 to 59 year old group, one was male, the other female.

Figure 2 shows the duration of arthritis.

The dosage ranges for the classification of high, moderate and low are found in Table I. These are ranges generally accepted by most rheumatologists and are the same as used by Black.

The relationships with dosage and duration of the steroid therapy is presented in Table II. One patient with P.S.C. had received a moderate dosage for eight years, the other had received low to moderate doses intermittently over a four year period. However, over the six month period before her eye examination she had been on about 40 mg of prednisone daily for an exacerbation of her arthritis, thus placing her in the high dosage group. Obviously, a series of only 2 patients with P.S.C. does not permit correlations to be drawn regarding duration and intensity of steroid therapy. Both patients had a family history of cataracts, a fact with clear implications. One patient had 20/60 visual acuity with correction and the other required cataract surgery on each eye.

The other ocular findings included congenital ptosis (1 case), keratitis sicca (2 cases), old chorioretinitis (1 case) but not in a patient with P.S.C., and cataracts. The cataract types were divided as follows: coronary 2, cerulean 1, cortical 2, nuclear 3, nu-

clear and cortical 3 and P.S.C. 2. A most interesting finding was that of an asymptomatic malignant melanoma picked up in the survey which may have gone many months before becoming apparent. The diagnosis was proven after enucleation.

Most of the arthritis patients were also treated with gold, salicylates and phenylbutazone at various times. All had received 5 to 10 x-ray examinations over the years. A few had received skull x-rays.

DISCUSSION

The lower incidence of posterior subcapsular cataracts in our series (5 percent) and that of Black (39 percent) requires explanation. This discrepancy between the two series is too great to occur by chance variation alone, is not explained by differences in management or nomenclature of the cataract types and therefore is probably due to differences in the patients themselves. The most likely difference is the origin of the patients. Ours were obtained from general medical practice and the arthritis clinic, whereas Black's were apparently referred to the N.I.H. It is evident that referral cases will inherently be a preselected, more seriously ill group of patients, and would be more likely to show complications of all types.

A continuation of this study is planned.

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HUMAN RETINAL NEURONS IN TISSUE CULTURE

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This report presents evidence that human retinal ganglion cells can be successfully grown and maintained in tissue culture. This is the first step towards a whole new field of important research concerning the reactions and the pathology of isolated living human retinal neurons.

MATERIAL AND TECHNIQUE

The cultures were obtained from human embryos in the fourth to fifth month of gestation and in one case from an enucleated eye of a four and a half month old child. The retinae were separated under aseptic conditions and pieces about 1 mm square were placed in a plasma clot consisting of 50 percent cockerel plasma and 50 percent chicken-embryo extract. Three pieces were placed on one slide and they were incubated in roller tubes at 37°C. The solution which was used for incubation consisted of 20 percent human serum, 2 percent chicken embryo extract, and Gay's balanced salt solution with 600 mg percent glucose and antibiotics. The culture slides were removed from the tubes and placed in perfusion chambers¹ at regular intervals, starting on the fourth day and continuing until the fourth month of culture. The cultures have been maintained in chambers for periods up to three months. While in chambers the cells were regularly photographed with still and time-lapse camera. Later they were fixed and stained with a modified Bodian silver technique. Since it was the main goal of the present investigation to study the morphological differentiation, addition of Cortisone (Geiger²) was avoided. Antibiotics which might also be ex-

pected to limit the life span of ganglion cells were not found to cause deterioration.

FINDINGS

Growing cultures of embryonal retinae reveal that the explant produces a very steady supply of cells which slowly migrate from it and form an undifferentiated monolayer of cells. These cells show poorly outlined individual borders, but easily identifiable nuclei. During the observation of this growth-zone three distinct zones are constantly seen in all the cultures (fig. 1).

1. The inner zone, which consists of polyhedral cells, is quite uniform. After approximately two to six weeks this zone becomes interwoven with fibers, which apparently have their origin in small multipolar cells. Although these cells with their processes have not been studied in detail, we believe that they represent connective tissue elements which form a supporting network.

2. The intermediate zone is characterized by homogenization of the cellular elements which in this area always lose the appearance of individual cells. Frequently we encounter an effect which seems to be of necrotizing nature. The homogenization is complete and the only surviving structures are the small apparently fibroblastic elements with their processes, and sometimes differentiated neuronal elements.

3. The outer zone consists as a rule of densely packed, parallel oriented elongated cells. From this zone the single cells proliferate and eventually they dissolve their connections with the explant.

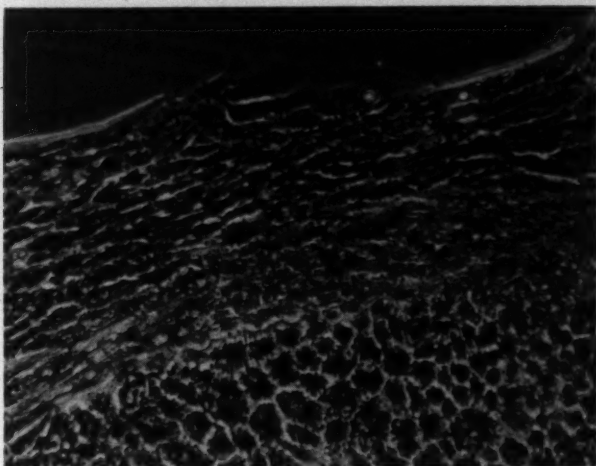
THE PATTERN OF OUTGROWTH IN EMBRYONAL MATERIAL

There are two basic forms of outgrowth which have been observed.

1. The cells on the surface of the outer zone proliferate, their cytoplasm becomes clearly outlined and their cell bodies en-

*From the Research Division, Columbus Psychiatric Institute and Hospital and the Department of Pathology, Ohio State University and from the Department of Ophthalmic Surgery, University of Michigan Medical Center, Ann Arbor, Mich. Supported in part by Grants No. B-3002 and B-2873 of the U.S. Department of Health, Education, and Welfare.

Fig. 1 (Liss and Wolter). Initial outgrowth with three zones of cells. Living culture. Phase.



larged. In this stage the cells display frequent mitotic divisions. These cells, in their pre-migrating stage, may appear as single cells (fig. 2). However, they also may be seen in small (fig. 3), or in large groups (fig. 4). Sometimes the large cell groups migrate away from the original explant. Others remain stationary until they eventually mature.

2. The second pattern of outgrowth may be called the organoid formation. In this pattern the proliferating cells of the outer zones form cellular extensions but do not

migrate. Some of these extensions unite with another outgrowth, producing a closed ring (fig. 5). We have observed in time-lapse photography and in still pictures taken at regular intervals that this effect is not a product of cytoplasmic contraction in the mono-layer. It represents reunification of two areas of cellular outgrowth which have been separated before. When the proliferation progresses, the ring remains within the outer layer of the growth zone (fig. 6) and sometimes borders the middle layer. The cellular behavior inside the ring is similar

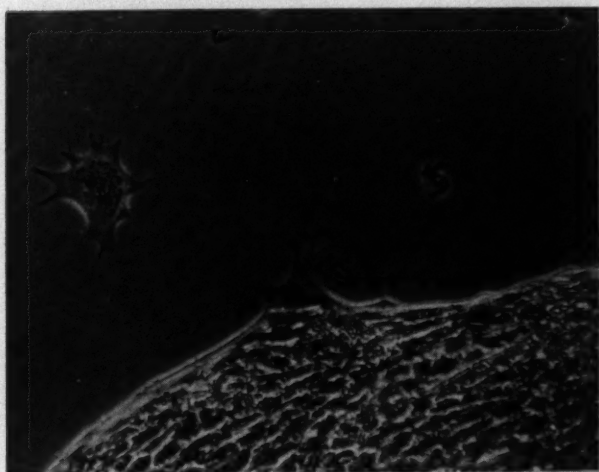


Fig. 2 (Liss and Wolter). Single cells which dissolve their connection with explant. Living culture. Phase.

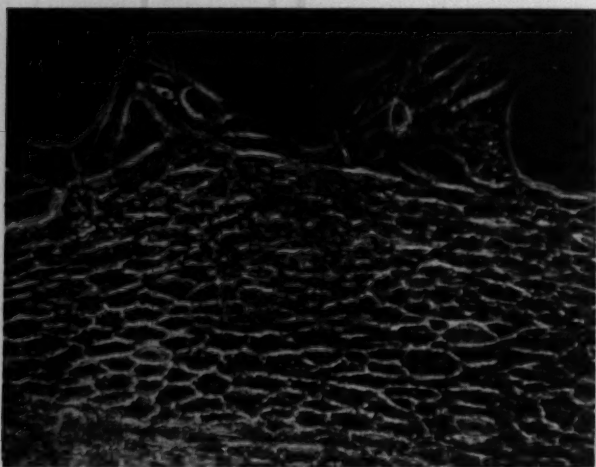


Fig. 3 (Liss and Wolter). Group of cells with beginning differentiation. Living culture. Phase.

to that observed in the outer zone on the periphery and causes sometimes subdivision of the ring in two smaller lumina. At the time the cultures were fixed for the staining, none of the cells in the ring had shown a distinct differentiation.

THE GROWTH PATTERN IN THE RETINAL TISSUE OF A CHILD

The growth of the pieces of retina of a four and a half month old child is different. The three growth zones are absent and the cells which migrate from the explant are of

three types: glial, ganglionic, and mesenchymal. In contrast to the embryonic cultures there is no maturation of the cells after migration. Divisions are frequent in glial cells and fibroblasts but no divisions are observed in the ganglion cells. Furthermore, the survival times of the ganglion cells are shorter in the cultures of infant retina than in embryonic retina. The pattern of behavior of our explants is quite similar to that of cat-cerebellum described by Pomerat and Costero.³ Although there are certain differences such as lack of a wide spectrum, dif-

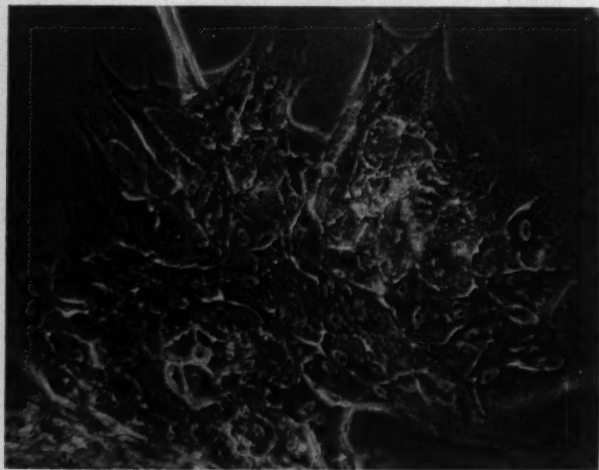


Fig. 4 (Liss and Wolter). Large group of cells as extension of mono-layer. Living culture. Phase.

Fig. 5 (Liss and Wolter). Early and progressed stage of "organoid formation." Living culture. Phase.

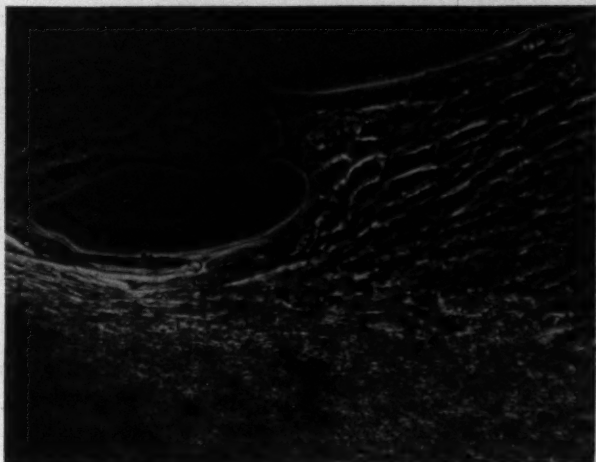


Fig. 6 (Liss and Wolter). Early and progressed stage of "organoid formation." Living culture. Phase.

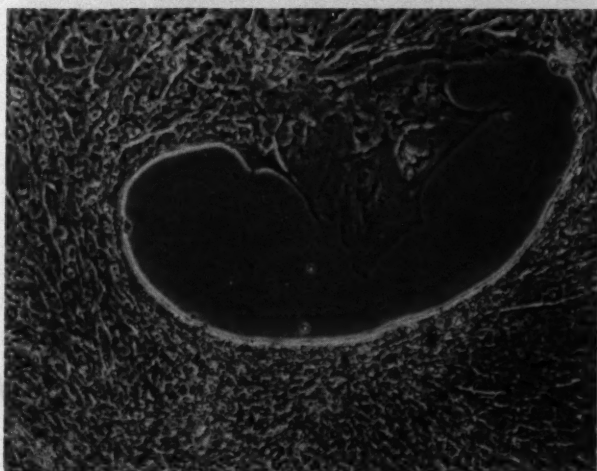
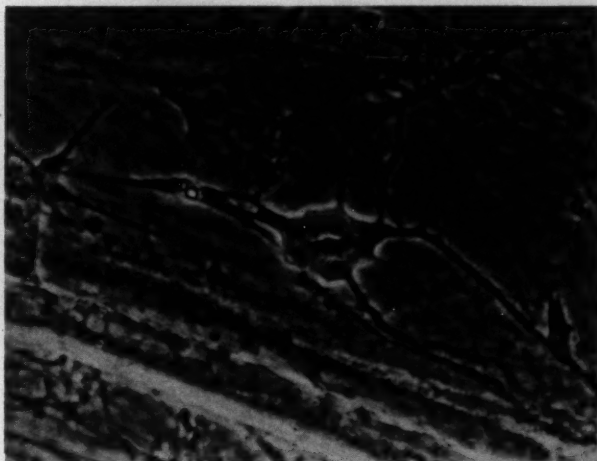


Fig. 7 (Liss and Wolter). Ganglion cells from the inner zone of the explant. Living culture. Phase.



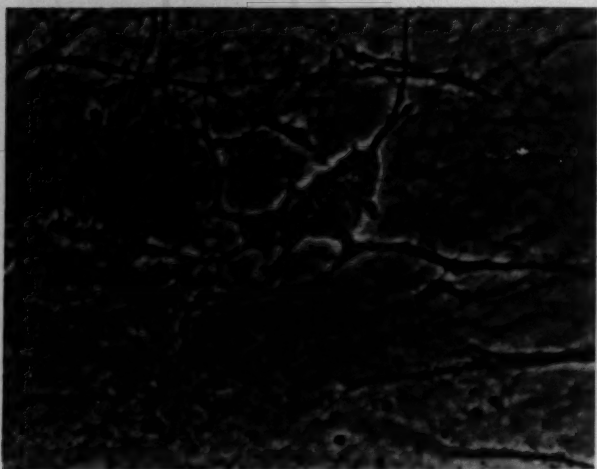


Fig. 8 (Liss and Wolter). Ganglion cells from the inner zone of the explant. Living culture. Phase.

ferent types of cells in the cultures, and the tendency of retinal ganglion cells to migrate away from the explant. The Purkinje cells, in contrast, always remain close to the explant.

THE CELL DIFFERENTIATION IN EMBRYONAL MATERIAL

In vitro the neuronal elements are seen to mature at the beginning of the third week. The cells which have reached the stage of morphological maturity remain stationary in their surroundings. They do not migrate, and no divisions are seen in these cells. They remain unchanged for a period up to three

and a half months. Degenerative changes start to develop early in the fourth month. The different types of mature ganglion cells are shown in Figures 7 to 10. They illustrate that human retinal ganglion cells can mature in various areas of the explant. The two neurons shown in Figures 7 and 8 are from the inner zone of the explant. The cell in Figure 7 has rounded to oval body and three main processes. The processes do not divide but give rise to perpendicular collaterals. The cell shown in Figure 8 has more processes than the one previously described and the collaterals are oriented perpendicular to the main process. In addition

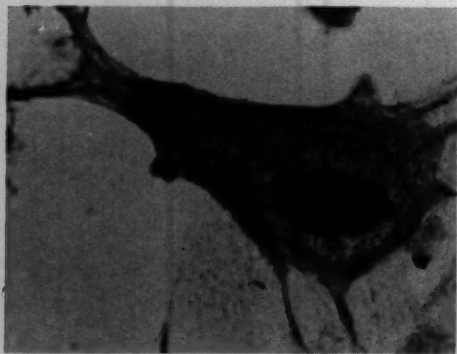


Fig. 9 (Liss and Wolter). Ganglion cell in the outer zone. Fixed culture. Bodian impregnation.

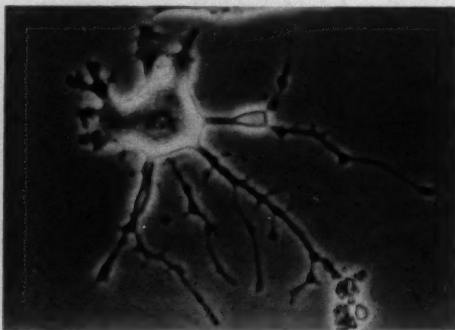


Fig. 10 (Liss and Wolter). Ganglion cell with dissolved connection with the explant. Living culture. Phase.

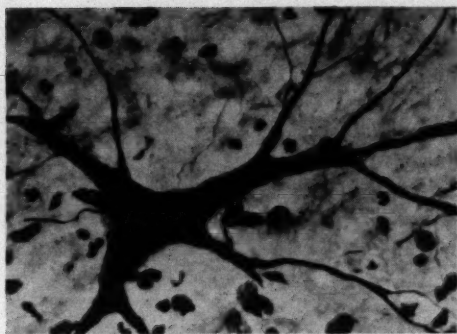


Fig. 11 (Liss and Wolter). Inner part of a large ganglion cell of the human retina seen in a flat section. Hortega stain.

there are divisions of processes in proximity to the cell body. In both these cells nuclei as well as the intracellular granules can be easily distinguished. The neuron shown in Figure 9 represents a cell migrating away from the explant which is about to dissolve its connection with the explant. It

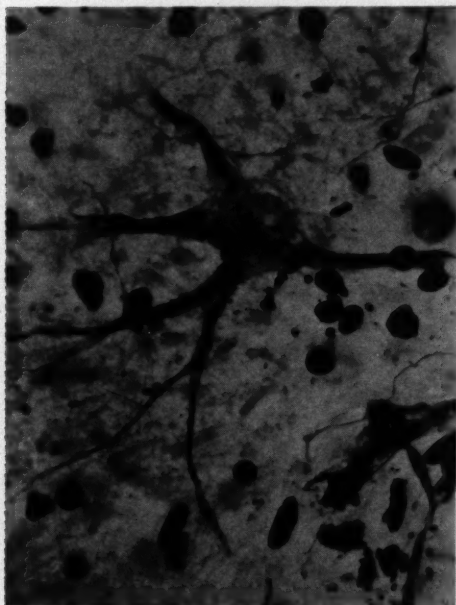


Fig. 12 (Liss and Wolter). Inner part of a ganglion cell of the human retina with its dendrites seen in a flat section. Hortega stain.

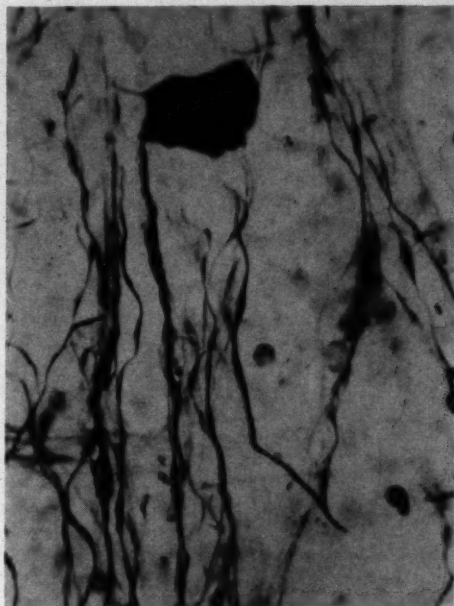


Fig. 13 (Liss and Wolter). Outer part of a ganglion cell of the human retina seen in a flat section. This part extends into the nerve fiber layer and sends its neurite towards the optic disk. Hortega stain.

is oval in shape and there are numerous processes, some of them retracted. Each of the processes gives numerous collateral branches. This cell is from a culture which was fixed and stained with modified Bodian technique. Distinct intracellular fibers and granules can be seen in this cell. It was apparently fixed before it completely dissolved its connection with the explant. The ganglion cell in Figure 10 migrated away from the explant and discloses no connection with other cells.

COMPARISON OF NEURONS SEEN IN TISSUE CULTURE TO NEURONS OF ADULT HUMAN RETINA

A comparison of the neurons grown in tissue culture to neurons of normal adult human retina is, of course, of great interest. Figures 11 to 15 show examples of different human retinal neurons as seen in flat sec-

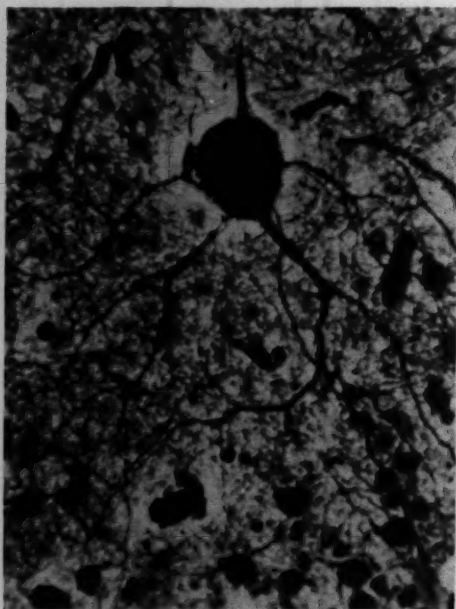


Fig. 14 (Liss and Wolter). Ganglion cell of the human retina in a transitional section. The neurite is seen on top of the cell body. Five dendrites are seen in the lower part of the picture. Hortege stain.

tions stained with the silver carbonate technique of Hortege.⁴ Figures 11 and 12 show the outer portions of large ganglion cells of the ganglion cell layer with the main branches of dendrites extending into the inner plexiform layer. Figure 13 shows the inner portion of a similar ganglion cell which extends into the nerve fiber layer and send its neurite towards the optic disk. Figure 14 shows the cell body of a smaller type of ganglion cell of the human retina. Its neurite is not in the plane of the flat section. Figure 15 gives an example of a so-called amacrine cell of the human retina.

The comparison of mature human retinal ganglion cells (figs. 11-15) to neurons grown in tissue culture of human retinal tissue (figs. 7-10) shows that the relatively mature cells in vitro somewhat resemble adult retinal neurons. However, the neurons in tissue culture do not show the extreme differentiation of the complicated elements

in the adult human retina. All the observed neurons in tissue culture have the basic appearance of the neurons of the inner retina. They have a large star-shaped body and a nucleolated nucleus. The branching cellular processes are much like the dendrites of the neurons of the inner retina. However, no neurites can be distinguished. No cells resembling bipolar cells or rods and cones have been positively demonstrated.

DISCUSSION

A large depot of undifferentiated cells which apparently represents neuroectodermal elements can be observed in the culture of human retina. The differentiation of ganglion cells proceeds slowly. It first becomes apparent in the second or third week after explanation and proceeds at a steady pace until the fourth month. The differentiated ganglion cells resemble in the general appearance primitive neurons of the inner

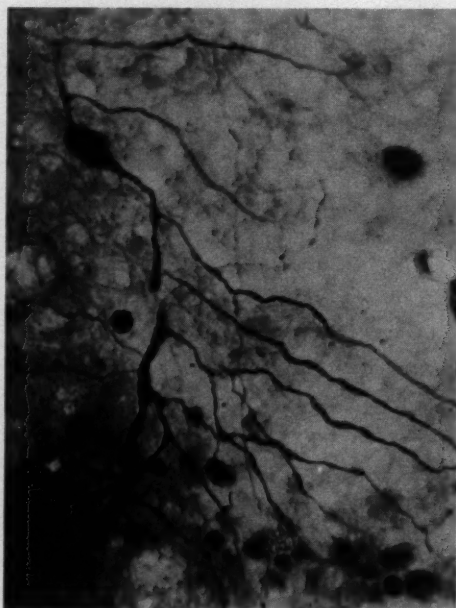


Fig. 15 (Liss and Wolter). A so-called amacrine cell of the human retina seen in a flat section. Hortege stain.

retinal layers of the adult retina. A closer classification is not possible. These cells never display any signs of morphological regression and apparently they have lost their ability for cellular division. Mitosis can be observed only in the undifferentiated matrix and never in the mature ganglion cells. After maturation in vitro degeneration occurs after several months of maintenance. The apparent attempt of organoid formation which bears morphological resemblance to the primitive tubular structure of neurogenic

tissues will require further study (compare Wolter⁵). Connective tissue cells are found among the neuroectodermal elements. They have a tendency to form a matrix in the intermediate zone of growth.

SUMMARY

Human retinal neurons can be grown in tissue culture. The mature retinal neurons in vitro resemble primitive ganglion cells of the inner retinal layers.

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THE NEUTRALIZATION OF CHOLINESTERASE INHIBITION BY VARIOUS OXIMES AND BY ATROPINE

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Earlier work by Harris and McCulloch¹ has shown that the oxime MINA (monoisnitrosoacetone) when given by topical application has an appreciable action, in rabbits, in counteracting the miotic effect of DFP (diisopropylfluorophosphate).¹ A 5 percent concentration was sufficient to give a significant action. The effect was noted when MINA was given from ½ hour before to 2 hours after DFP was applied. Once a decrease in miosis was established it lasted throughout the period of action of the DFP. Further work has now been completed, comparing the action of four other oximes with that of MINA. P₂S (soluble methane sulfonate of pyridine-2-aldoxime) was found to be the best² and has been tested against phospholine iodide and demecarium bromide. Further, the effect of atropine has been tested against DFP, separately from, and in conjunction with, P₂S.

The oximes were obtained in powder or crystalline form and were prepared in 5 percent concentrations, firstly in buffered sodium hypophosphate and, secondly, in 1:10,000 Zephiran solution. The solutions were freshly made before each day's experiments. DFP was used as a 0.05 percent solution in dried peanut oil. Phospholine iodide 0.25 percent and demecarium bromide 0.25 percent were used in solution as supplied by the manufacturer. Atropine sulphate was used in a 2 percent solution.

As in previous work,¹ 5 rabbits were used for each part of the experiment. The miotic was instilled into both conjunctival sacs of the animals. The oxime was placed in the conjunctival sac of the right eye to assess the effect of oxime on miotic, while the left eye was used as a control to assess the effect of the miotic alone. Photographs to show pupillary size were taken at the start of each

trial and at intervals during the experiment as shown in Figures 1 and 2 and in Table 1.

The method of photography was described previously;¹ all pupillary sizes are recorded as a percentage of the initial pupillary size. The results presented represent the average of five eyes for each point.

RESULTS

1. Oxime 5 percent in buffer solution.

a) P_2S was less effective than the other oximes used, but it was non-irritating. Figure 1 shows the charted response and illustrates the smooth curve typical of the reversal of miosis obtained with each of the oximes. The results obtained one hour after the drugs were instilled have been arbitrarily selected to illustrate the immediate maximal response in the right eye, and they also represent the low point of the curves: for the right eye 51 ± 4.4 percent, for the left eye 42 ± 3.9 percent.

b) DAM was more effective than P_2S but it was slightly irritating: right eye 65 ± 3.7 percent, left eye 48 ± 5.9 percent.

c) 2-oximino-3-pentanone was slightly more effective than DAM but it was more irritating: right eye 68 ± 8.2 percent, left eye 49 ± 4.3 percent.

d) 3-oximino-2-pentanone was extremely irritating and proved to be an unreliable drug.

2. Oxime 5 percent in 1:1000 Zephiran solution.

a) MINA, DAM, and 2-oximino-3-pentanone did not show any improvement. The curves obtained were similar to those in Figure 1.

b) P_2S showed an increased effectiveness sufficient to make it the most promising of the oximes tested. The curve obtained for the right eye (fig. 2) shows a steeply rising slope immediately following the initial fall, indicating a more prompt and effective reversal of miosis. The results obtained one hour after the drugs were instilled do not, in this case, represent the low points of the curves, but they do illustrate the immediate maximal response for comparison with previous results: right eye 58 ± 7.2 percent, left eye 37 ± 1.5 percent.

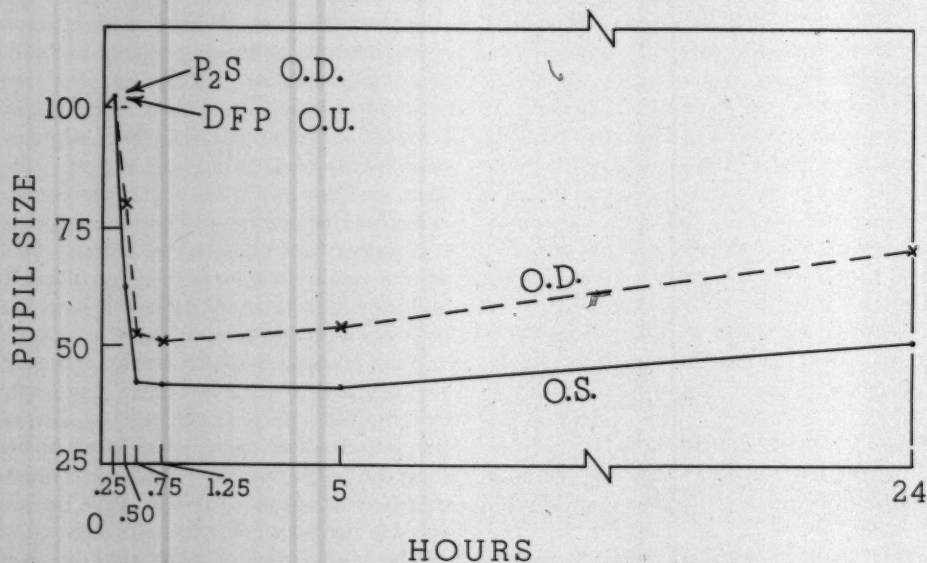


Fig. 1 (Hunter and McCulloch). P_2S 5 percent in buffer solution produced a moderate reversal of miosis. The character of the curve is typical of that obtained with the oximes.

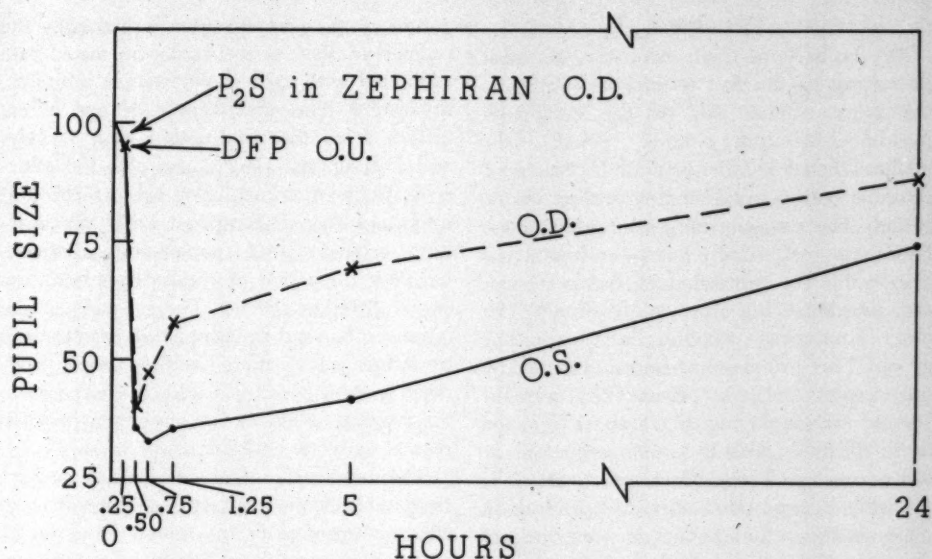


Fig. 2 (Hunter and McCulloch). P₂S 5 percent in 1:10,000 Zephiran solution of the oximes tested produced the most prompt and effective reversal of miosis.

3. P₂S 5 percent in 1:10,000 Zephiran was then tested against phospholine iodide and demecarium bromide, producing smooth curves similar to those in Figure 1. Of the two, a better reversal of miosis was observed with the former, but in neither case was the action as marked as that against DFP. Results obtained one hour after the drugs were instilled were: phospholine iodide right eye 54 ± 6.8 percent, left eye 44 ± 8.3 percent; demecarium bromide right eye 68 ± 7.3 percent, left eye 61 ± 10.2 percent.

4. The action of atropine on DFP miosis was assessed by instilling it in combination with 5 percent P₂S in 1:10,000 Zephiran, and alone, in the right eye.

a) P₂S and DFP were given as outlined in Table 1; atropine 2 percent was placed in the right conjunctival sac $\frac{1}{2}$ hour before, $\frac{1}{2}$ hour after, and 2 hours after the miotics in 3 separate trials. In each case there was an immediate mydriasis in the right eye which was maintained over the duration of effect of the DFP. The high points of the curves being 123 ± 6.1 percent, 111 ± 7.6 percent, and 113 ± 9.2 percent, respectively.

The left eye showed a miosis, the low points of the curves being 44 ± 8.4 percent, 37 ± 6.8 percent, and 47 ± 9.0 percent, respectively.

b) Atropine 2 percent instilled into the right conjunctival sac $\frac{1}{2}$ hour before, $\frac{1}{2}$ hour after and 2 hours after DFP had been placed in both eyes produced similar results. The high points of the curves for the right eyes were 120 ± 8.1 percent, 127 ± 5.3 percent and 106.76 percent respectively, while the low points for the left eyes were 42 ± 7.5 percent, 45 ± 9.8 percent and 37 ± 3.1 percent, respectively.

TABLE 1

Time	Photograph	Drug
-5 minutes		
0		oxime
5		oxime
10		oxime
15	"	
15		miotic
30	"	
45	"	
75	"	
5 hours	"	
24 hours	"	

DISCUSSION

On the basis of their chemistry, the most promising of the five oximes is P_2S . It is the more soluble salt of the compound pyridine-2-aldoxime, while PAM (the methiodide salt) is the less soluble salt. P_2S contains both a pyridine ring and an oxime group. The oxime group, which characterizes these compounds, has been considered responsible for protection of cholinesterase, the pyridine ring for reactivation.² The other four drugs contain only an oxime group. This promise has been borne out by our results; once its penetrability was increased by employing Zephiran, P_2S acted more efficiently than the other oximes.

Enzyme protection and reactivation is probably a complicated activity depending on many factors; the amounts of inhibitor and oxime present, their relative efficiency, and the amount of cholinesterase necessary for normal muscle fibre response. It has been suggested that in man the improvement obtained by oximes in organophosphate poisoning is not completely due to enzyme protection and reactivation.³ Although work completed recently delineating more clearly the chemistry and biochemistry of the oxime group has been of assistance in clinical investigation,⁴ oxime action is not completely explained.

One of the complications in assessing the oximes is that each has to be tested and evaluated with each cholinesterase inhibitor, separately. The effectiveness of one oxime differs from that of another (e.g. MINA and P_2S against DFP), and also the effectiveness of an oxime differs against different inhibitors (e.g. P_2S against DFP, demacarium bromide and phospholine iodide). Further, the action of these drugs is different in different species. The selection of the oxime to be used in ophthalmic practice will be influenced by these factors, and a prognosis as to their clinical effectiveness has to be guarded when based on either in vitro results or work on laboratory animals.

The mydriasis obtained with 2 percent atropine indicates that, in the rabbit, it is an effective antidote to the miosis from DFP, whether given with an oxime or not. When given with an oxime there was a slightly more prompt reversal of pupillary action.

CONCLUSIONS

1. P_2S 5 percent in 1:10,000 Zephiran solution is a partial antidote to miosis due to cholinesterase inhibitor, in rabbits.
2. Atropine 2 percent has a marked mydriatic effect on the rabbit's pupils, in the presence of DFP.

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FURTHER STUDIES ON THE AUTONOMIC-LIKE PROPERTIES OF OCULAR EXTRACTS*

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Previous investigations in this Laboratory have shown the presence of autonomic-like properties in iris and ciliary body preparations.¹ Similar results with iris preparations have also been reported by Ambache.² These preparations are atropine resistant and were shown to be neither histamine or serotonin. The parasympathomimetic effects of these extracts in smooth-muscle bath preparations seemed to be caused by a fatty acid-like substance. It seemed judicious to seek other manifestations of these parasympathomimetic properties. The blood pressure reducing properties of some of these extracts are thus being reported in this paper. The results obtained thus far are suggestive of distinctive depressor effects of extracts of sphincter and dilator pupillae.

METHODS

1. The apparatus designed for measuring depressor activity is similar to that described in the USP apparatus section.³ This is a recording mercury manometer with a 1:1 displacement of blood pressure changes. The depressor assay itself is also described in the USP Biological Assay section.⁴ Because of the exceedingly small amount of extract substance available, rats were used for the assay. The rat is relatively insensitive to histamine, therefore this latter substance could not be used for a parasympathomimetic comparison assay. The rats were treated with a 1 gm per liter dibenzylene solution (0.02 mg per 100 gm body weight). This drug was administered intramuscularly 12 hours prior to testing. The animals were anesthe-

tized with 0.4 ml per 100 gm body weight of 25 percent urethane injected intraperitoneally. When the jugular cannulation was completed 0.5 ml of a 10 mg per cc heparin solution was injected.

2. Tissue dissections and extract preparations were as described previously.¹

3. Injection procedure.

The total volume of each of the intrajugular injections was 0.4 ml. The tissue was usually prepared such that each milliliter of the final extract contained the active substance from two grams of tissue. The necessary aliquot from this solution was diluted to 0.4 ml with 0.9 percent saline for injection purposes. The tissue weights described in the results represent, therefore, the amount of tissue-equivalent used for that particular injection.

RESULTS

1. The relative depressor effects of varying amounts of dilator and sphincter pupillae are shown graphically in Figure 1. These results compare favorably to what has been observed in similar experiments. Because, as is shown with the control saline injections, there is a definite pressor effect due to the volume of each injection, and because the latency for the onset of this pressor effect is essentially the same as the latency following tissue extract administration, actual depressor effects of the tissue extracts are of a relatively high order of magnitude.

2. Figure 2 shows the latency of response, and duration of action of both the saline controls and tissue extracts. It is to be noted that the depressor action with sphincter pupillae extract is maintained for a much longer period of time than with dilator pupillae extracts. Also, at similar extract concentration, the magnitudes of change with the sphincter preparation is about 50

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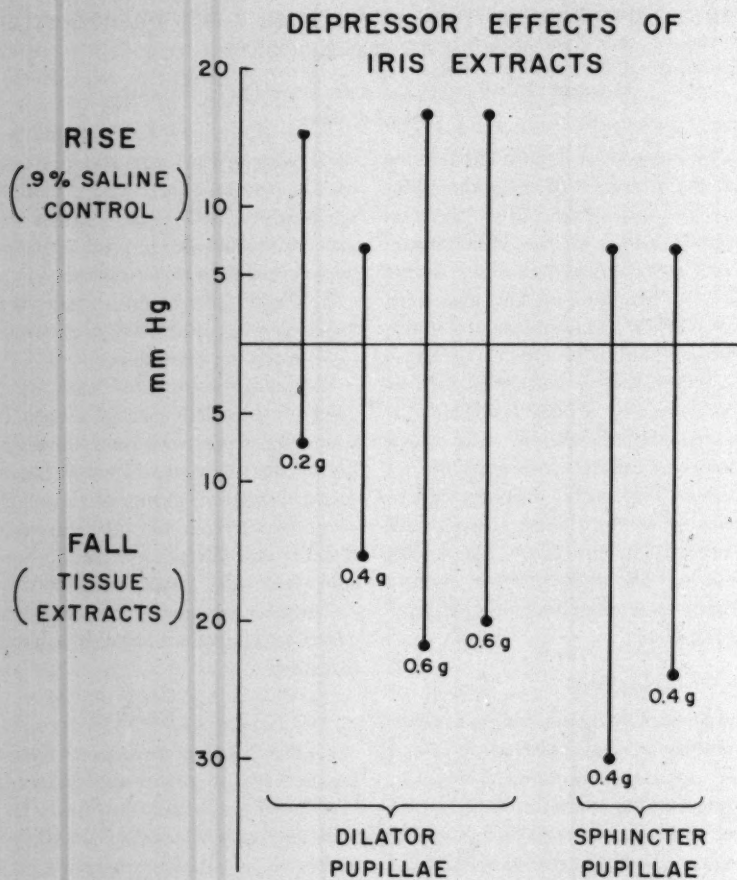


Fig. 1

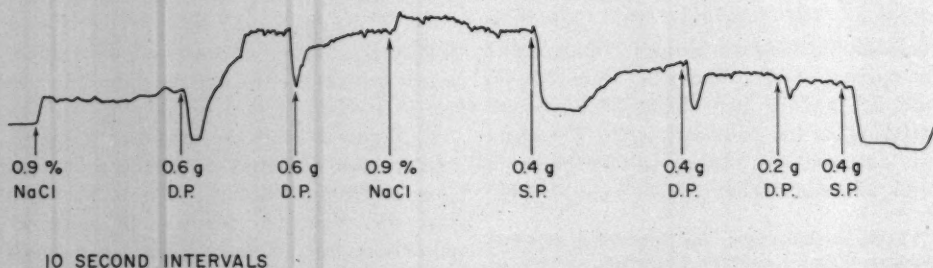


Fig. 2

percent greater. An average 37 mm of mercury drop in blood pressure per gram original wet weight of dilator tissue is seen in Figures 1 and 2.

DISCUSSION

The parasympathomimetic activity of iris extracts is again shown in these experiments. The material prepared from sphincter pupillae appears to be a longer acting and more potent substance than the material of the dilator pupillae. There was almost immediate recovery from the dilator extract-induced changes. It is possible that a material from one tissue site is transported and converted into a different form at another tissue site. This latter possibility must be considered due to the fact that we are undoubtedly dealing with chemically similar substances from these two tissue sources (similar extraction procedures employed). The fact that dilator pupillae preparations appear more active than sphincter extracts in a muscle bath system¹ lends an even greater interest to the differences in depressor effects seen with these two tissues.

Further investigations on both the chemical nature of the active substances and their biological roles are being conducted.

SUMMARY

1. Blood pressure-lowering effect of extracts of hog sphincter pupillae and dilator pupillae are described.

2. The depressor effect from the sphincter is almost double and is maintained for a much longer period of time when compared with the dilator effects.

Department of Ophthalmology.

ADDENDUM

Subsequent studies show a *very marked* pupil-constriction effect in rabbits when iris or dilator preparations from hog eyes are injected into #10 polyethylene tubing which had been surgically placed in the posterior chamber of the rabbit eyes (several weeks prior to experiments). The tubing was brought to the outside and could drain freely at will. Details are being prepared for a future publication.

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FURTHER STUDIES OF CILIARY PROCESS ENZYME SYSTEMS*

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Previous investigations in this laboratory have shown the presence of oxidative phosphorylation in ciliary process tissue.¹ Adenylic Acid (AMP) deamination activity

in ciliary process extracts has also been described.^{2,3} In at least one of these deaminases marked activation in the presence of adenosinetriphosphate (ATP) is observed.³ Adenosinediphosphate (ADP) can also activate the AMP-deaminase in terms of myokinase-like reactions in which quantities of ATP and AMP are released. The studies being reported here relate to other adenine nucleotide synthesis and deaminating se-

*From the Laboratory for Research in Ophthalmology, Western Reserve University, School of Medicine, and the University Hospitals of Cleveland. This study was supported in part by Research Grant B-121 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health.

ADENOSINE PHOSPHORAMIDE

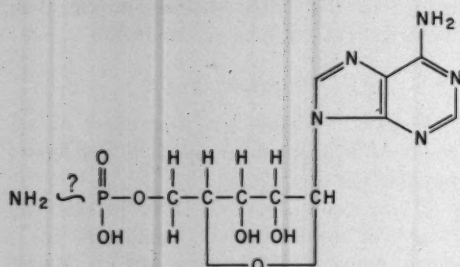


Fig. 1

quences as well as to a pyrophosphatase system.

Katunuma⁴ has indicated a means by which ATP or ADP may be synthesized. The intermediate for this synthesis was an adenosine phosphoramidate (AMP-NH₂) (fig. 1) and inorganic pyrophosphate. From investigations in this laboratory it would seem clear that ATP and ADP syntheses do, in fact, take place when AMP-NH₂ is incubated with pyrophosphate in the presence of acetone powder preparations of hog ciliary

process tissue. Pyrophosphatase activity was also observed during this incubation.

METHODS

1. Hog ciliary process tissues were collected and acetone powder extracts prepared.² Ten ml of acetone per gm. of original wet weight tissue were used in each of 3 successive mortar and pestle grindings (dry-ice temperature). When enough acetone powder (which was stored in a sealed container at -12°C) was accumulated the extract was made by grinding a 1 percent powder in water with a Teflon pestle in a glass homogenizer and centrifuging at 105,000 g. for 30 minutes. The supernatant of this was studied.

2. Conway Cup incubations and analytical procedures for ammonia determinations have been described previously.²

3. Analytical procedures for nucleotide determinations were also as described previously.^{1,2}

4. Amino acids were determined according to the ninhydrin test described by Spies.⁵

TABLE 1
SYNTHESIS OF ATP AND ADP IN CILIARY PROCESS EXTRACTS

Substrate (micro-moles)		Micro-moles Recovered After 40 Min. Incubation					Micro-moles Total PO ₄ Added (A)	Micro-moles Total PO ₄ Recovered (B)	A/B
Pyro-PO ₄	Adenosine Phosphoramidate	Ortho-PO ₄	Pyro-PO ₄	ATP	ADP	Adenosine Phosphoramidate and/or AMP			
0.5	10	1.95	—	—	0.38	10.34	11	13.05	0.84
0.5	10	1.94	—	—	0.38	8.22	11	10.92	1.01
5.0	10	10.04	—	—	2.28	8.38	20	20.70	0.97
5.0	10	10.34	—	—	2.33	8.59	20	23.59	0.85
20	10	2.35	16.81	1.97	0.39	lost	50	?	?
20	10	2.65	15.60	1.83	0.29	9.76	50	49.68	1.01
20	10 (AMP)	2.66	17.30	—	—	11.82	50	49.08	1.02
20	10 (AMP)	2.21	14.42	—	—	11.10	50	42.15	1.19
20	—	3.02	15.72	—	—	—	40	34.46	1.16
20	—	3.27	15.91	—	—	—	40	35.09	1.14
none	10	1.06	—	—	—	9.68	10	10.74	0.93
none	10	1.03	—	—	—	8.26	10	9.29	1.08
0.5*	10	—	—	—	0.16	7.6	10	7.76	1.29
20†	10	—	16.39	0.40	—	9.83	50	43.81	1.14
20†	10	—	14.58	0.27	—	9.16	50	39.13	1.28

* Boiled enzyme.

† No enzyme.

Contents: 0.05M Tris; 0.05M Succinate; 0.0075M KCl; 0.006M MgCl₂; 0.04M NaCl; 0.11% Protein; final pH 7.4; incubations at 21°C.

TABLE 2
DEAMINATION OF ADENOSINE PHOSPHORAMIDE BY
CILIARY PROCESS EXTRACTS

Substrate (micro-moles)		NH ₃ Released (micro-moles)
Adenosine Phosphoramidate	Pyrophosphate	
10	none	1.17
10	0.5	1.22
10	5.0	1.16
10	20	0.73
10*	20	0
10 (AMP)	20	0.27
none	20	0

* No enzyme.

Contents: 0.05M Tris; 0.05M Succinate; 0.0075M KCl; 0.006M MgCl₂; 0.04M NaCl; 0.11% Protein; final pH 7.4; incubations at 21°C.

RESULTS

ATP and ADP Synthesis: The need for pyrophosphate in the presence of AMP-NH₂ and ciliary process extracts in order that ATP or ADP be synthesized is shown in Table 1. Non-enzymatic synthesis in trace amounts can also take place. It is clear that the pyrophosphate must be such that its concentration is greater than AMP-NH₂ in order that a relatively high yield of ATP can take place. This has been corroborated in other experiments. On the other hand, stoichiometrically smaller amounts of pyrophosphate result in a high yield of ADP.

Pyrophosphatase Activity: Activity of pyrophosphate-splitting enzyme resulting in orthophosphate accumulation is also shown in the data of Table 1.

Adenosine Phosphoramidate Deaminase Activity: The adenosine phosphoramidate used as a substrate in these reactions is shown in Figure 1. It is noted that the ammonia attached to the phosphate of adenylic acid is hydrolyzed in the systems being reported here. The ammonia of the purine nucleus did not appear at any time to be split from the molecule. It is this latter ammonia which is split in the adenylic acid deaminase reactions studied previously^{2,3} resulting thereby in the formation of inosinic acid. Table 2 shows that ammonia release is not dependent on the presence of pyrophosphate although

if the pyrophosphate concentration is double that of the AMP-NH₂ concentration then there is a reduction in the quantity of ammonia accumulated. Pyrophosphate in high concentration also seems to reduce the AMP deamination which would be normally greater than is shown in this Table.^{2,3} The reduced ammonia output in the vessels containing 20 micro-moles of the pyrophosphate could be a reflection either of enzyme inhibition or ammonia release coupled with some type of transamination reactions.

Transamination Study: Attempts to demonstrate transamination of the NH₂ of AMP-NH₂ to alpha-ketoglutaric acid substrate by accumulation of glutamic acid have thus far been without success.

DISCUSSION

The results consistently indicate that either ADP or ATP can be synthesized from pyrophosphate and AMP-NH₂ in the presence of an enzyme or enzymes of hog ciliary process tissue. The stoichiometric relationship between the pyrophosphate and AMP-NH₂ seems a deciding factor as to which of these polyphosphate nucleotides will be synthesized. Adenosine phosphoramidate deaminase activity is demonstrated in these studies. This activity is reduced in the presence of high concentration of pyrophosphate. The data show, too, some interesting pyrophosphatase properties of ciliary process extracts.

The balance studies concerned with total added phosphorus and total recovery of this phosphorus are shown in Table 1.

Studies will be made on the role of myokinase-like reactions in this systems. As these systems are studied further it will be of great interest to know, too, if transamination reactions are present.

SUMMARY

When acetone powder extracts of hog ciliary process tissues were prepared the following reactions were observed:

1. Synthesis of ATP or ADP in the pres-

ence of adenosine phosphoramidate and inorganic pyrophosphate.

2. Pyrophosphatase splitting of inorganic pyrophosphate.

3. Deamination of the ammonia attached to the phosphorus of adenosine phosphoramidate. This reaction is not dependent on the presence of inorganic pyrophosphate.

Attempts to demonstrate transamination activity have been unsuccessful so far.

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Department of Ophthalmology.

ADDENDUM

Subsequent experiments, using column chromatography analysis, show, also, an accumulation of inosine and adenosine. The details of the formation of these compounds from very stable substances will be discussed in a future publication.

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